

FIGURE 1A

1 CGGATGCTGC TGCTACTGTC ACTTCTGCCG CTGCCGCTGT TGTTACAGAT
 51 TTTGCTTTG CTCCTTCTAC CGCATGACAA TTGTTTCCT CGCCTAAGCA
 101 GATACCAGCC TCAGATGCTC AAGGTGAGAG TCTTGCCTTT CGCTCTGGGC
 151 TATTGGTTCA CTTAATCCGG TCAATTGTT CGCTGCTCGT GGTTGTCTTT
 201 CTCCCCGCC CTCCTCCCCC TGTTTGTTT TGTTTCGCTT GCTTCGGGG
 251 GGACGCTCCT TCCCTCAGTC AGAAGAGCTG GAATTGCTTG AGAGGCGTAT
 301 AAGGAATTAT AAAAGTGGCC AGGAAACACG AGCGCAGTGA CTGCAGAGCT
 351 GCCCTTGGCT TCGGCAAGGC AGCGTGAGCG GCAGAGGGCT CGGGCAGGGG
 401 GCGGGGGGTC TCCTTTTCC CGTGC GTTCC TCTTCTCCA GTTCGGATGA
 451 TGTTGCTGTT TCGGACCTCT CGCTGACTCC TGCCCTGTGA TTTTGCTGA
 501 GCGCTGTGAC TGTTACTCCG TCTCTTCTG TCTGTGTTTC ACAGTAATGG
 551 ACTGTGATAG AGTTAAGGCC TTTGGAGGT GAGCTGTGTC ACAGCTGATG
 601 CTTAACATG TCTGAAGTAG GCACCGAGAC TTTCCCCAGC CCCTCGGCTC
 651 AGCTGAGCCC TGATGCATCC CTTGGCGGGC TCCCGGCTGA GGAGAACATG
 701 CCGGGGCCCC ACAGAGAGGA CAGCAGGGTC CCAGGTGTGG CAGGCCTGGC
 751 CTCGACCTGC TCGGTGTGCC TGGAAAGCAGA GCGACTGAAG GGCTGCCTCA
 801 ACTCTGAGAA GATCTGCATC GCCCCTATCC TGGCTTGCCT GCTCAGCCTC
 851 TGCCTCTGCA TTGCTGGCCT CAAGTGGTC TTTGTGGACA AGATTTTGA
 901 GTATGACTCT CCTACACACC TTGACCTGG GAGGATAGGA CAAGACCAA
 951 GGAGCACTGT GGATCCTACA GCTCTGTCTG CCTGGGTGCC TTCGGAGGTG
 1001 TATGCCTCAC CCTTCCCCAT ACCTAGCCTT GAGAGCAAGG CTGAAGTGAC
 1051 AGTGCAAAC T GACAGCTCGC TCGTGCCTC CAGGCCCTTC CTTCAGCCTT
 1101 CTCTCTACAA CCGCATCCTA GATGTGGGT TGTGGTCCTC TGCCACACCG
 1151 TCACTGTCAC CATCCTCCCT GGAGCCTACC ACGGCATCTC AGGCACAAGC
 1201 AACAGAAACC AATCTCCAAA CTGCTCCAAA ACTTCCACT TCTACATCTA
 1251 CAACTGGGAC AAGTCATCTC ACAAAATGTG ACATAAAGCA GAAAGCCTTC
 1301 TGTGTAAATG GGGGAGAGTG CTACATGGTT AAAGACCTCC CAAACCCCTCC

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FIGURE 1B

1351 ACGATAACCTA TGCAGGTGCC CAAATGAATT TACTGGTGAT CGCTGCCAAA
1401 ACTACGTAAT CGCCAGCTTC TACAAGCATC TTGGGATTGA ATTTATGGAA
1451 GCTGAGGAAC TGTACCAGAA ACAGGGTGCTG ACCATAACTG GCATTTGCAT
1501 TGCTCTTCTA GTAGTTGGCA TCATGTGTGT GGTGGCCTAC TGCAAAACCA
1551 AGAACAGCAGAG GAAAAAGTTG CATGACCGCC TTCGGCAGAG CCTTCGCTCA
1601 GAGAGGAACA ACGTTATGAA CATGGCAAAT GGGCCACACC ACCCCAACCC
1651 ACCACCAGAC AATGTCCAGC TGGTGAATCA GTACGTTCA AAAAACATAA
1701 TCTCCAGTGA ACGTGTGTT GAGCGAGAAA CCGAGACCTC GTTTTCCACA
1751 AGCCACTACA CCTCAACAAAC TCATCACTCC ATGACAGTCA CCCAGACGCC
1801 TAGCCACAGC TGGAGTAATG GCCATACCGA AAGCATTCTC TCCGAAAGCC
1851 ACTCCGTGCT CGTCAGCTCC TCAGTGGAGA ATAGCAGGCA CACCAGCCC
1901 ACAGGGCCAC GAGGCCGCCT CAATGGCATT GGTGGGCCAA GGGAAAGGCAA
1951 CAGCTTCCTC CGGCATGCAA GAGAGACCCC TGACTCCTAC CGAGACTCTC
2001 CTCACAGTGA AAGGTATGTC TCAGCTATGA CCACACCAGC TCGCATGTCA
2051 CCCGTTGATT TCCACACTCC AACTTCTCCC AAGTCCCCTC CATCTGAAAT
2101 GTCACCACCA GTTTCAGCT TGACCATCTC CATCCCTCG GTGGCGGTGA
2151 GTCCCTTAT GGACCGAGGAG AGACCGCTGC TGTTGGTGAC CCCACCACGG
2201 CTGCGTGAGA AGTACGACAA CCACCTTCAG CAATTCAACT CCTTCCACAA
2251 CAATCCCACC CATGAGAGCA ACAGTCTGCC ACCCAGTCCT CTGAGGATAG
2301 TGGAGGATGA AGAGTATGAG ACCACGCAGG AGTACGAACC AGCACAGGAG
2351 CCTCCAAAGA AACTCACCAA CAGCCGGAGG GTGAAAAGAA CAAAGCCCAA
2401 TGGCCATATT TCCAGCAGGG TAGAAGTGGA CTCCGACACA AGCTCTCAGA
2451 GCACTAGCTC TGAGAGCGAA ACAGAAGATG AAAGAATAGG TGAGGATACA
2501 CCATTTCTTA GCATACAAAA TCCCATGGCA ACCAGTCTGG AGCCAGCCGC
2551 TGCATATCGG CTGGCTGAGA ACAGGACTAA CCCGGCAAAT CGCTTCTCCA
2601 CACCAGAAGA STTGCAAGCA AGGTTGTCCA GTGTAATAGC TAACCAAGAC
2651 CCTATTGCTG TATAAGACAT AAACAAAACA CATAGATTCA CATGTAAAAC

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FIGURE 1C

2701 TTTATTTAT ATAATGAAGT ATTCCACCTT TAAATTAAAC AATTTATTTT
2751 ATTTTAGCAA TTCCGCTGAT AGAAAACAAG AGTGGAAAAA GAAACTTTA
2801 TAAATTAAGT ATACGTATGT ACAAAATGTGT TATGTGCCAT ATGTAGCAAT
2851 TTTTACAGT ATTTCCAAAA TGGGGAAAGA TATCAATGGT GCCTTTATGT
2901 TATGTTATGT TGAGAGCAAG TTTTGACAG CTACAATGAT TGCTGTCCCG
2951 TAGTATTTG CAAAACCTTC TAGCCCTCAG TTGTTCTGGC TTTTTGTGC
3001 ATTGCATTAT AATGACTGGA TGTATGATTT GCAAGAATTG CAGAAGTCCC
3051 CATTGCTTG TTGTGGAATC CCCAGATCAA AAAGCCCTGT TATGGCACTC
3101 ACACCCTATC CACTCACCA GGAAAAAAAA AAAATCAAAA AAAAAAAA
3151 AAAAAAAAGA AAAGAAAGAG AAAAAAGAAA AGAAAAAGAA AAAAAAAGCT
3201 GAAAAAAATAA AA

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FIGURE 2

1 GCCCYCHFCR CRCCYRFCFC SFYRMTIVFL A*ADTSLRCS R*ESCLSLWA
 51 IGSLNPVNLF AARGCLSPRP PSPCFVLFRL LSGGRSFPQS EEELELLERRI
 101 RNYKSGQETR AQ*LQSCPWL RQGSVSGRGL GQGAGGLLFP VRSSSPSSDD
 151 VAVSDLSSLTP AL*FLLSAVT VTPSLSVCVS Q*WTVIELRP FGGECHS*C
 201 LNMSEVGTE FSPSPSAQLSP DASLGGLPAE ENMPGPHRED SRVPGVAGLA
 251 STCCVCLEAE RLKGCLNSEK ICIAPILACL LSLCLCIAGL KWVFVDKIFE
 301 YDSPTHLDPG RIGQDPRSTV DPTALSAWVP SEVYASPFPI PSLESKAEVT
 351 VQTDSSLVPS RPFLQPSLYN RILDVGLWSS ATPSLSPSSL EPTTASQAQA
 401 TETNLQTAPK LSTSTSTTGT SHLTKCDIKQ KAFCVNGGEC YMVKDLPNPP
 451 RYLCRCPNEF TGDRCQNYVM ASFYKHLGIE FMEAEELYQK RVLTITGICI
 501 ALLVVGIMCV VAYCKTKKQR KKLHDRLRQS LRSERNNVMN MANGPHHPNP
 551 PPNVQLVNQ YVSKNIIISSE RVVERETETS FSTSHYTSTT HHSMTVTQTP
 601 SHSWSNGHTE SILSESHSVL VSSSVENSRH TSPTGPRGRL NGIGGPREGN
 651 SFLRHARETP DSYRDSPHSE RYVSAMTTPA RMSPVDFHTP TSPKSPPSEM
 701 SPPVSSLTIS IPSVAVSPFM DEERPLLVT PPRLREKYDN HLQQFNSFHN
 751 NPTHESNSLP PSPLRIVEDE EYETTQEYEP AQEPPKKLTN SRRVKRTKPN
 801 GHISSRVEVD SDTSSQSTSS ESETEDERIG EDTPFLSIQN PMATSLEPAA
 851 AYRLAENRTN PANRFSTPEE LQARLSSVIA NQDPIAV*DI NKTHRFTCKT
 901 LFYIMKYSTF KLNNLFYFSN SADRQEWKK KLL*IKYTYV QMCYVPYVAI
 951 FYSISKMGKD INGAFMLCYV ESKFCTATMI AVP*YFAKPS SPQLFWLFCA
 1001 LHYNDWMYDL QELQKSPFAC CGIPRSKSPV MALTPYPLHQ EKKKIKKKKK
 1051 KKRKEREKRK EKEKKS*KNK

DECODED SEQUENCES

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FIGURE 3

1 CGGCCTGTAA GATGCTGTAT CATTGGTTG GGGGGCCTC TGCCTGGTAA
51 TGGACCGTGA GAGCGGCCAG GCCTTCTTCT GGAGGTGAGC CGATGGAGAT
101 TTATTCCCCA GACATGTCTG AGGTGCCGC CGAGAGGTCC TCCAGCCCCT
151 CCACTCAGCT GAGTGCAGAC CCATCTCTTG ATGGGCTTCC GGCAGCAGAA
201 GACATGCCAG AGCCCCAGAC TGAAGATGGG AGAACCCCTG GACTCGTGGG
251 CCTGGCCGTG CCCTGCTGTG CGTGCCTAGA AGCTGAGCGC CTGAGAGGTT
301 GCCTCAACTC AGAGAAAATC TGCATTGTCC CCATCCTGGC TTGCCTGGTC
351 AGCCTCTGCC TCTGCATCGC CGGCCTCAAG TGGGTATTTG TGGACAAGAT
401 CTTTGAATAT GACTCTCCTA CTCACCTTGA CCCTGGGGGG TTAGGCCAGG
451 ACCCTATTAT TTCTCTGGAC GCAACTGCTG CCTCAGCTGT GTGGGTGTCG
501 TCTGAGGCAT ACACCTCACCC TGTCTCTAGG GCTCAATCTG AAAGTGAGGT
551 TCAAGTTACA GTGCAAGGTG ACAAGGCTGT TGTCTCCTTT GAACCATCAG
601 CGGCACCGAC ACCGAAGAAT CGTATTTTG CCTTTCTTT CTTGCCGTCC
651 ACTGCGCCAT CCTTCCCTTC ACCCACCCGG AACCCCTGAGG TGAGAACGCC
701 CAAGTCAGCA ACTCAGCCAC AAACAACAGA AACTAATCTC CAAACTGCTC
751 CTAAACTTTC TACATCTACA TCCACCACTG GGACAAGCCA TCTTGTAAAA
801 TGTGCGGAGA AGGAGAAAAC TTTCTGTGTG AATGGAGGGG AGTGCTTCAT
851 GGTGAAAGAC CTTCAAACC CCTCGAGATA CTTGTGCAA CGCGGAGGAG
901 CTGTACCAGA AGAGAGTGCT GACCATAACC GGCATCTGCA TCGCCCTCCT
951 TGTGGTCGGC ATCATGTGTG TGGTGGCCTA CTGCAAAACC AAGAACAGC
1001 GGAAAAAGCT GCATGACCGT CTTCGGCAGA GCCTTCGGTC TGAACGAAAC
1051 AATACGATGA ACATTGCCAA TGGGCCTCAC CATCCTAACCC CACCCCCCGA
1101 GAATGTCCAG CTGGTGAATC AATACGTATC TAAAAACGTC ATCTCCAGTG
1151 AGCATATTGT TGAGAGAGAA GCAGAGACAT CCTTTCCAC CAGTCACTAT
1201 ACTTCCACAG CCCATCACTC CACTACTGTC ACCCAGACTC CTAGCCACAG
1251 CTGGAGCAAC GGACACACTG AAAGCATCCT TTCCGAAAGC CACTCTGTAA
1301 TCGTGATGTC ATCCGTAGAA AACAGTAGGC ACAGCAGCCC AACTGGGGCC
1351 G

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FIGURE 4

1 ACKMLYHLVG GASAW*WTVR AARPSSGGEP MEIYSPDMSE VAAERSSSPS
51 TQLSADPSLD GLPAAEDMPE PQTEDGRTPG LVGLAVPCCA CLEAERLRGC
101 LNSEKICIVP ILACLVSLCL CIAGLKWFV DKIFEYDSPT HLDPGGLGQD
151 PIISLADATAA SAVWVSSEAY TSPVSRAQSE SEVQVTQGD KAVVSFEPSA
201 APTPKNRIFA FSFLPSTAPS FPSPTRNPEV RTPKSATQPQ TTETNLQTAP
251 KLSTSTSTTG TSHLVKCAEK EKTFCVNGGE CFMVKDLSNP SRYLCKGGGA
301 VPEESADHNR HLHRPPCGRH HVCGGLLQNQ ETAEKAA*PS SAEPSV*TKQ
351 YDEHCQWASP S*PTPRECPA GESIRI*KRH LQ*AYC*ERS RDILFHQSLY
401 FHSPSLHYCH PDS*PQLEQR TH*KHPFRKP LCNRDVIRRK Q*AQQPNWG

BIOLOGICAL DATA

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FIGURE 5A

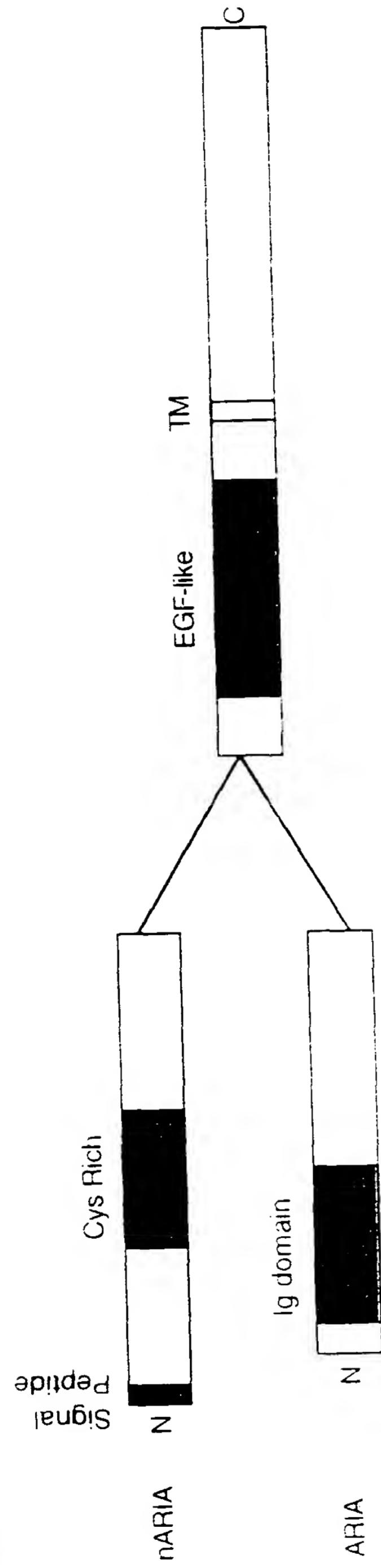
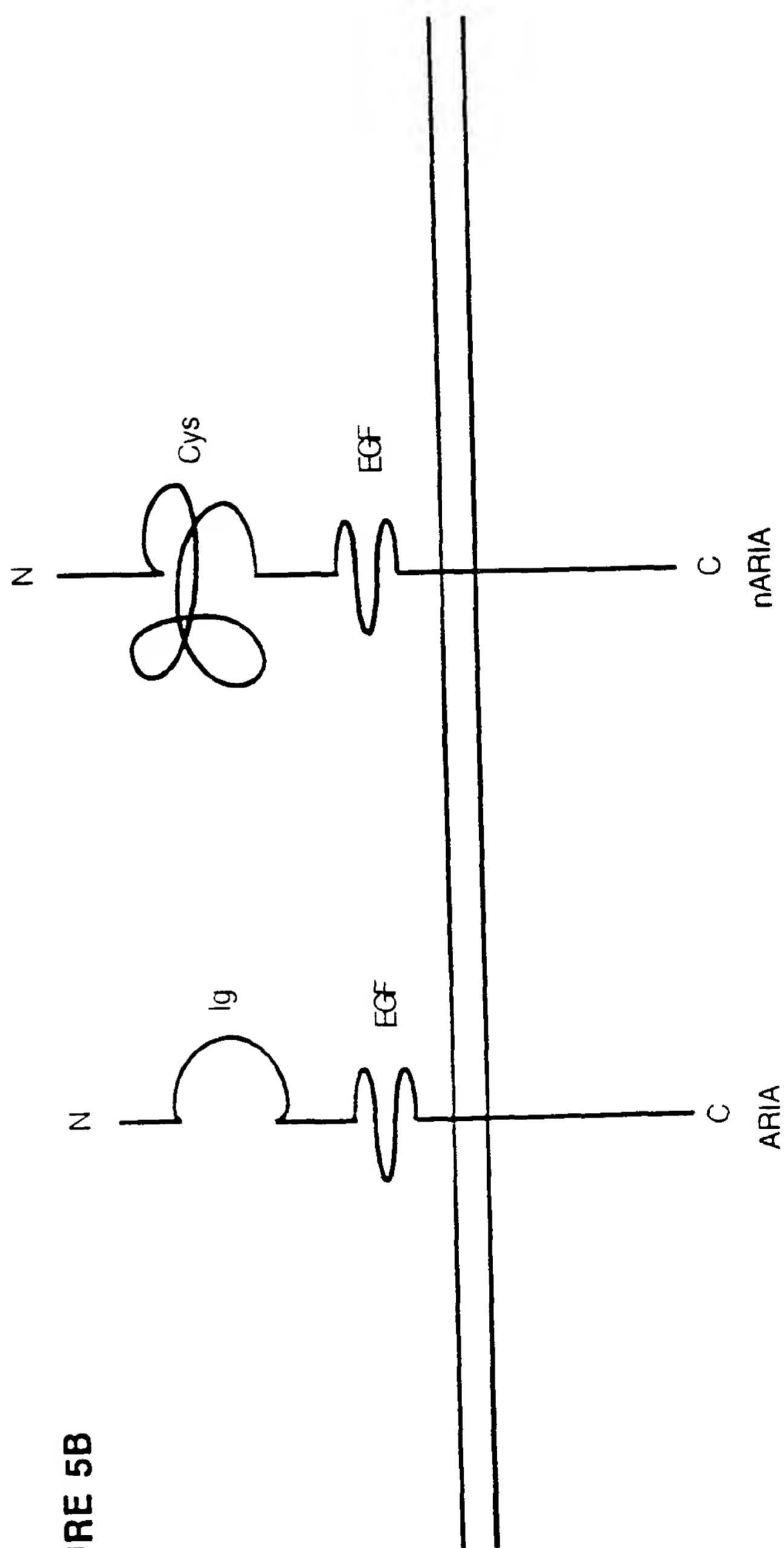
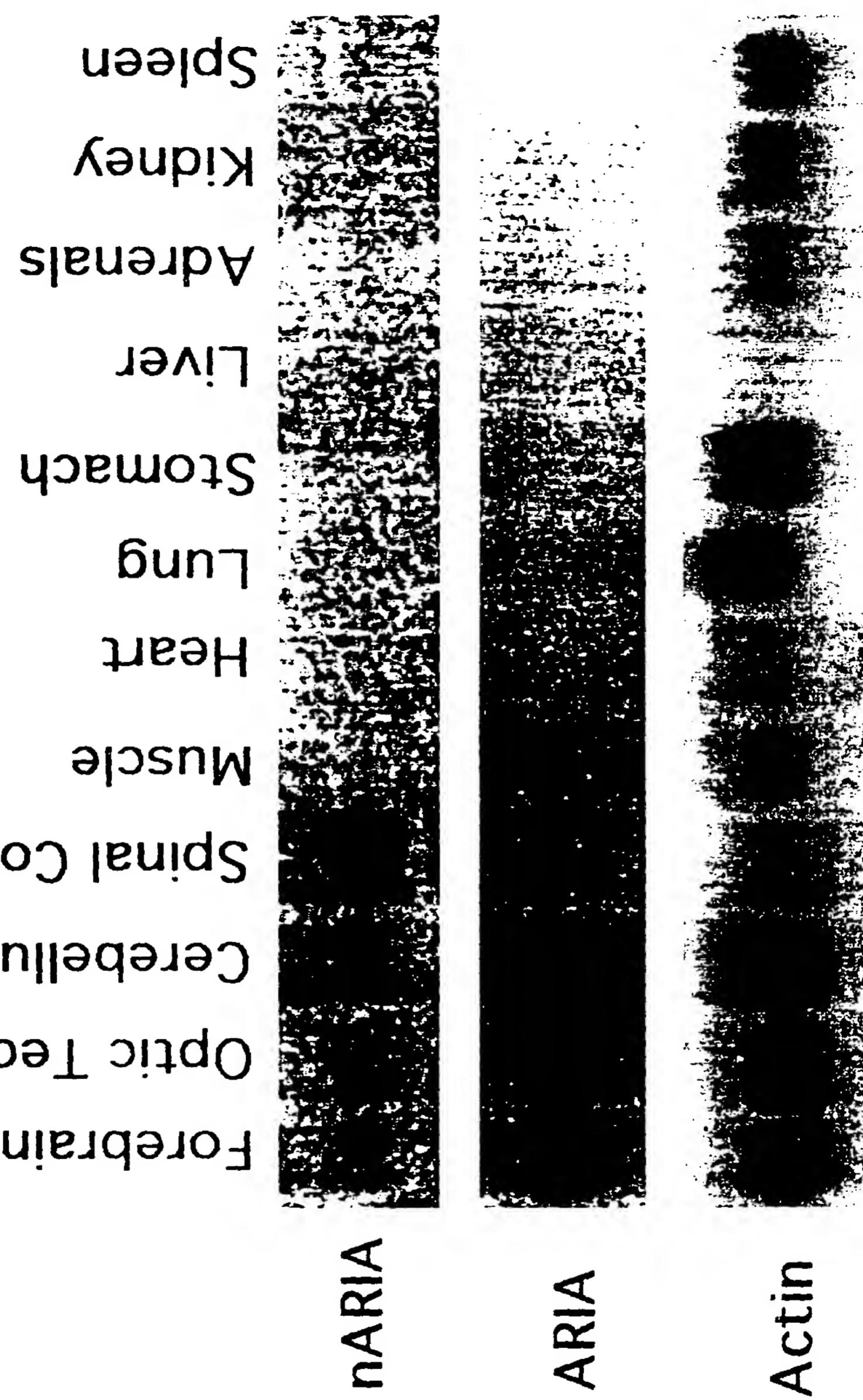


FIGURE 5B



0 2 4 6 8 10 12 14 16 18

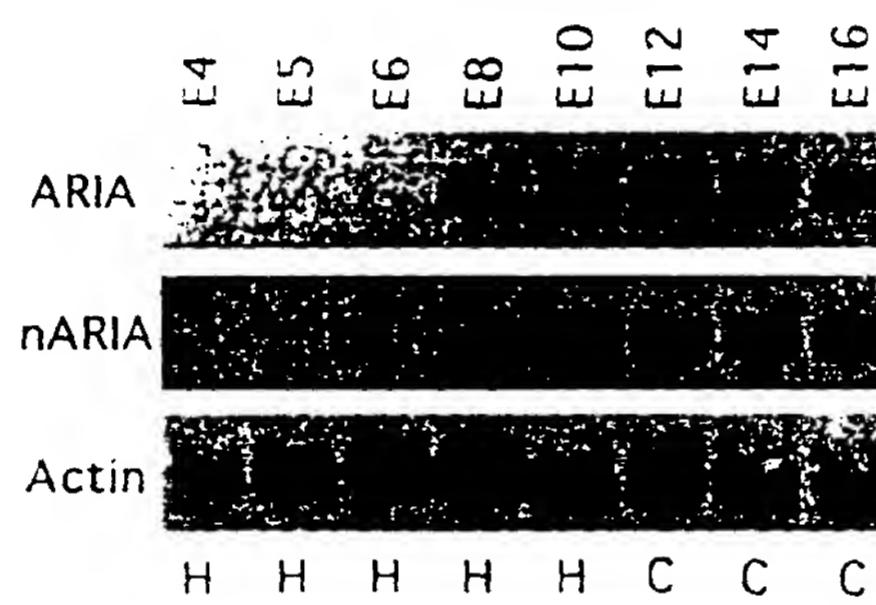
FIGURE 6



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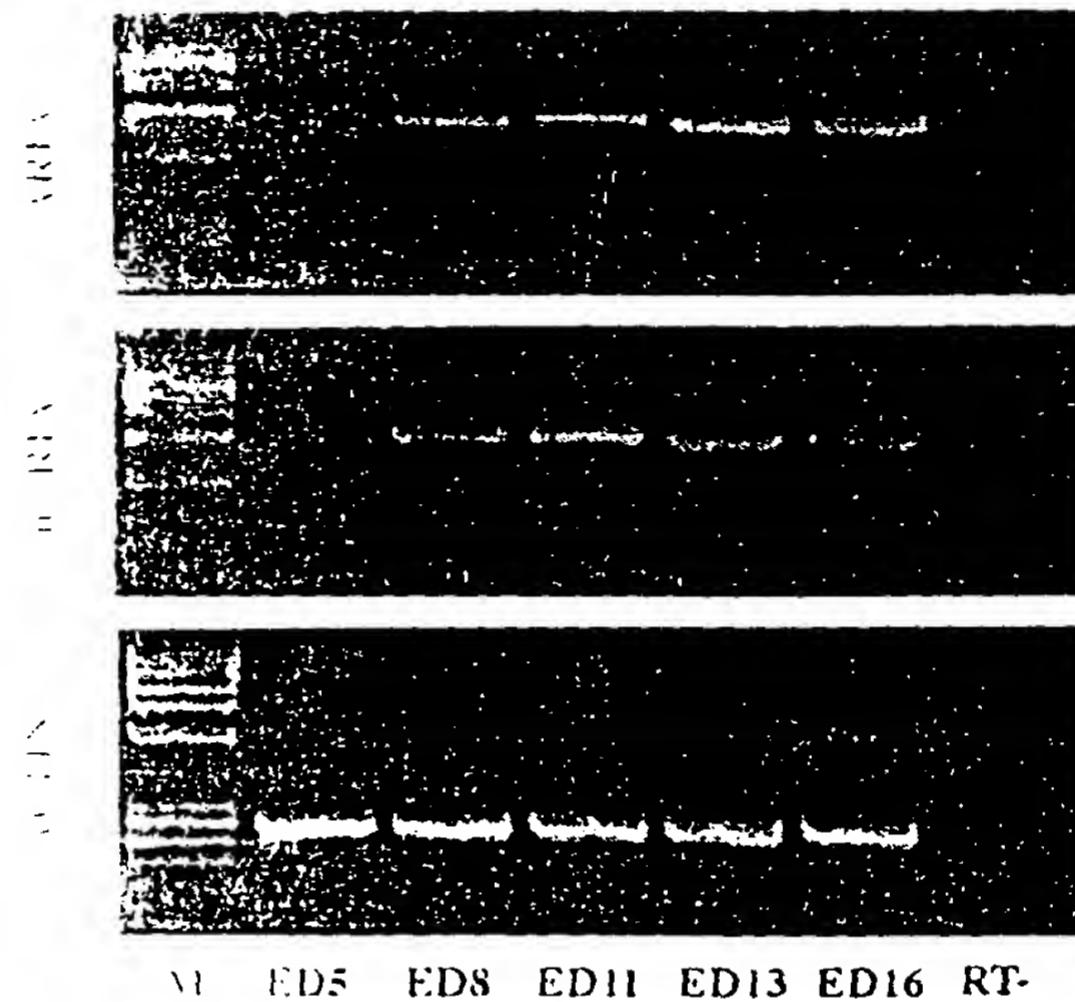
FIGURE 7

Developmental Northern of ARIA
and nARIA in the chick hindbrain
and cerebellum



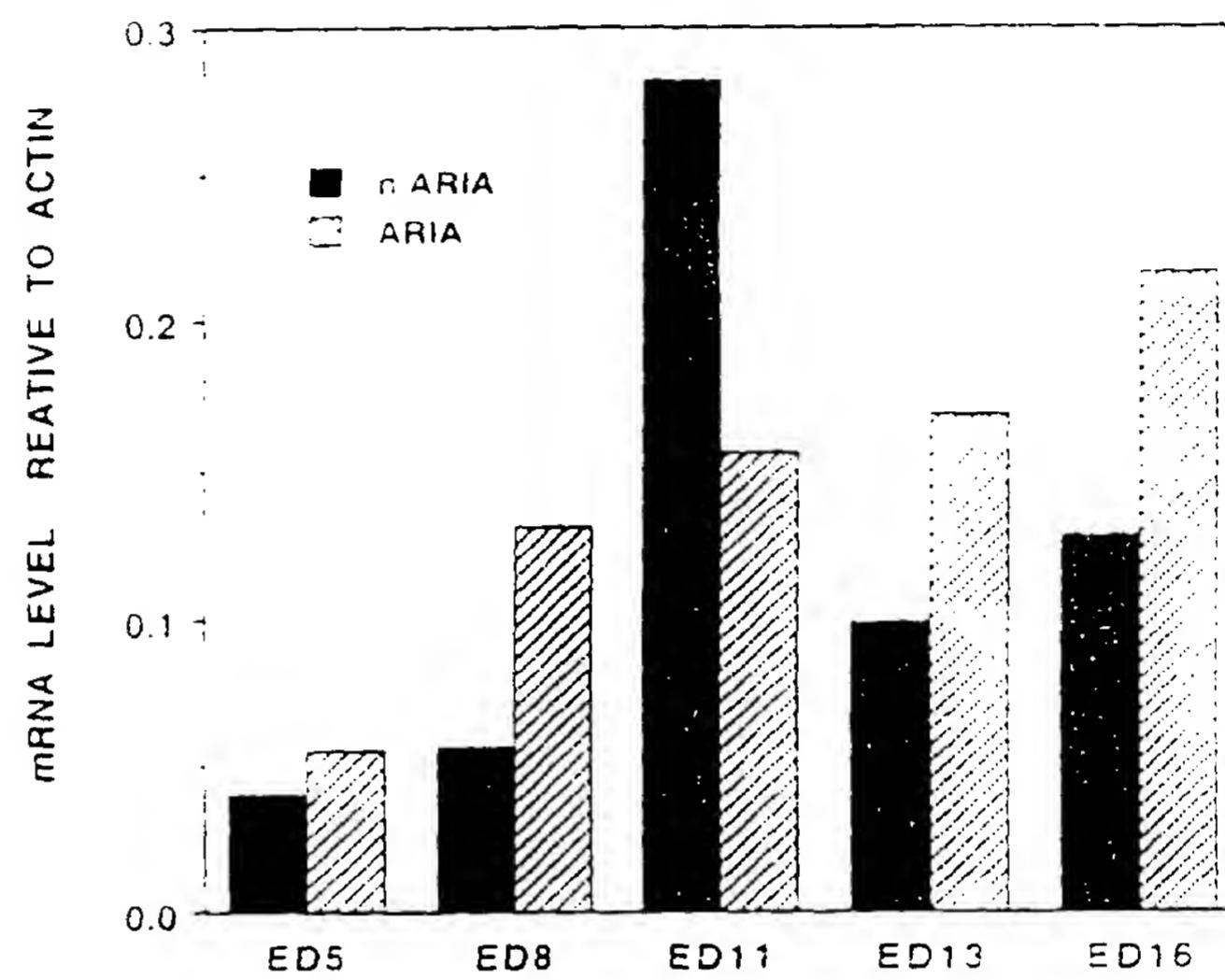
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FIGURE 8A



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FIGURE 8B



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FIGURE 9A

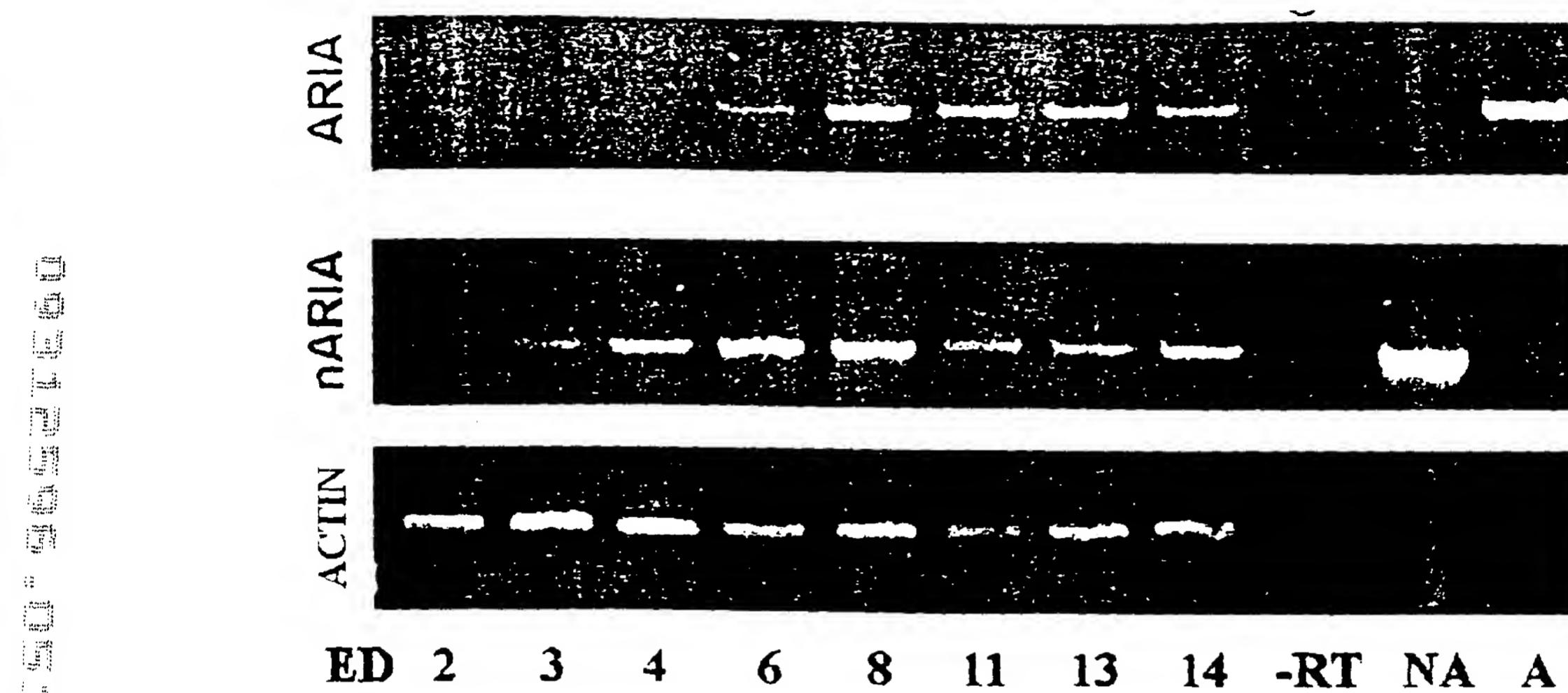
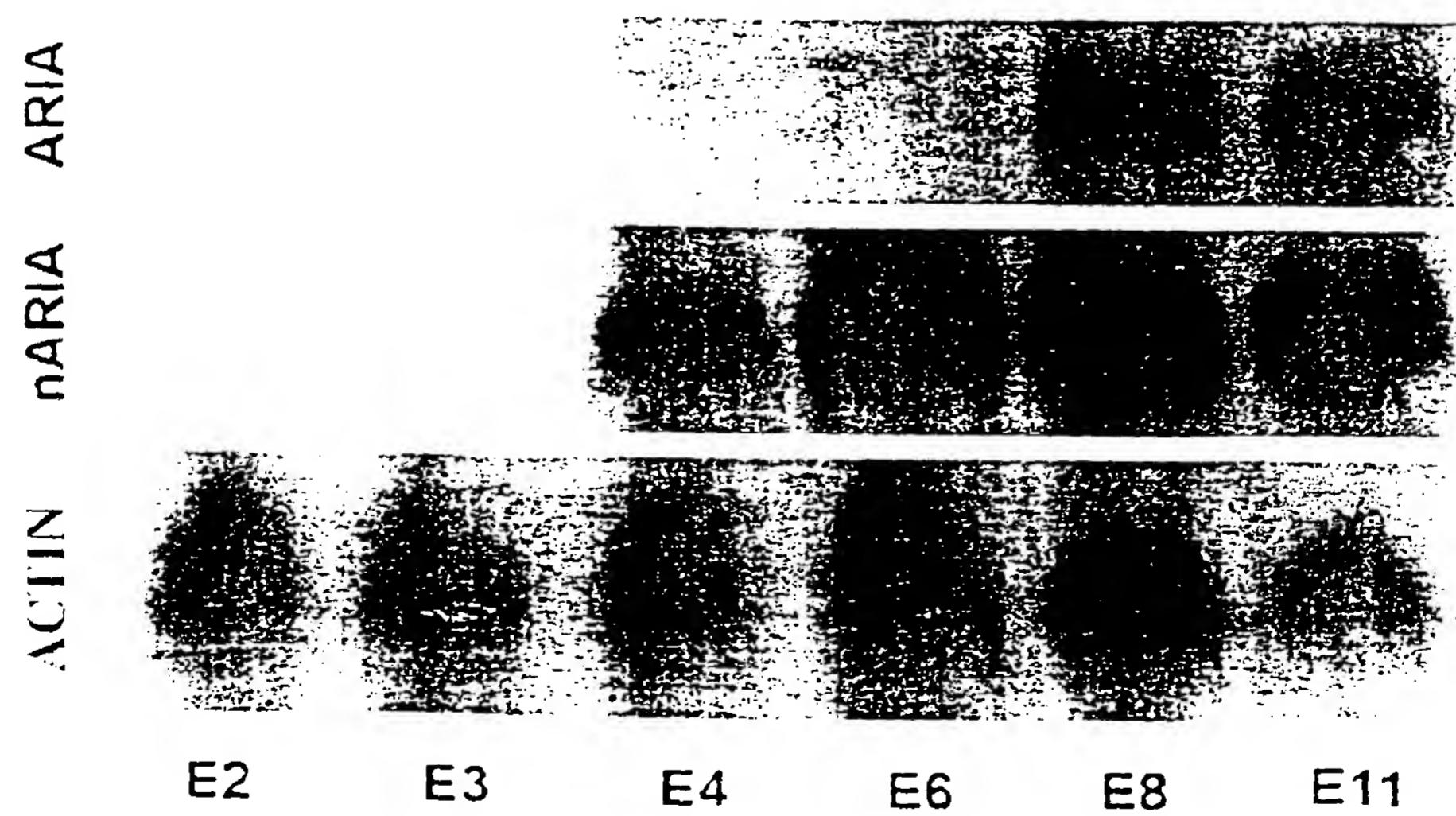
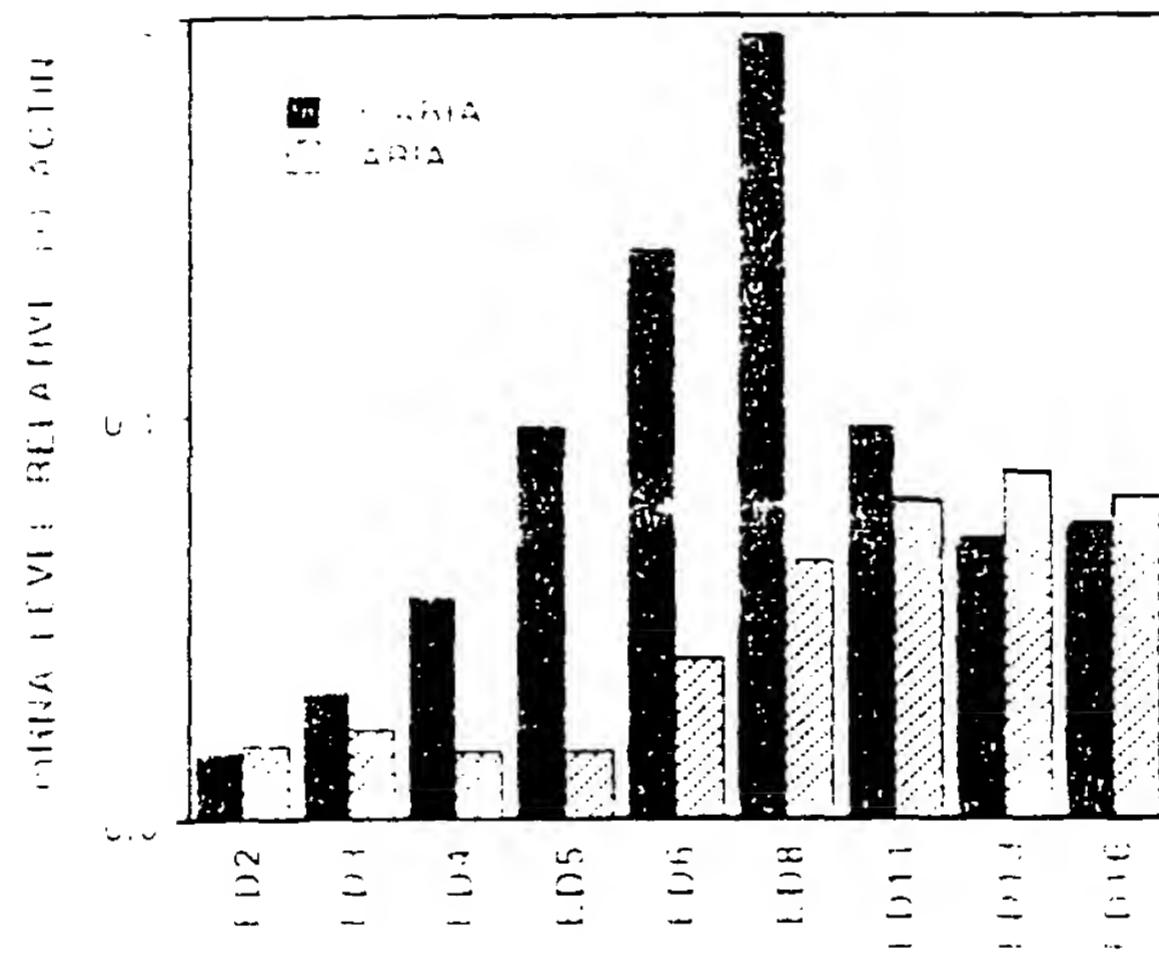


FIGURE 9B



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FIGURE 9C



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FIGURE 10A
ED5 trunk cross-section

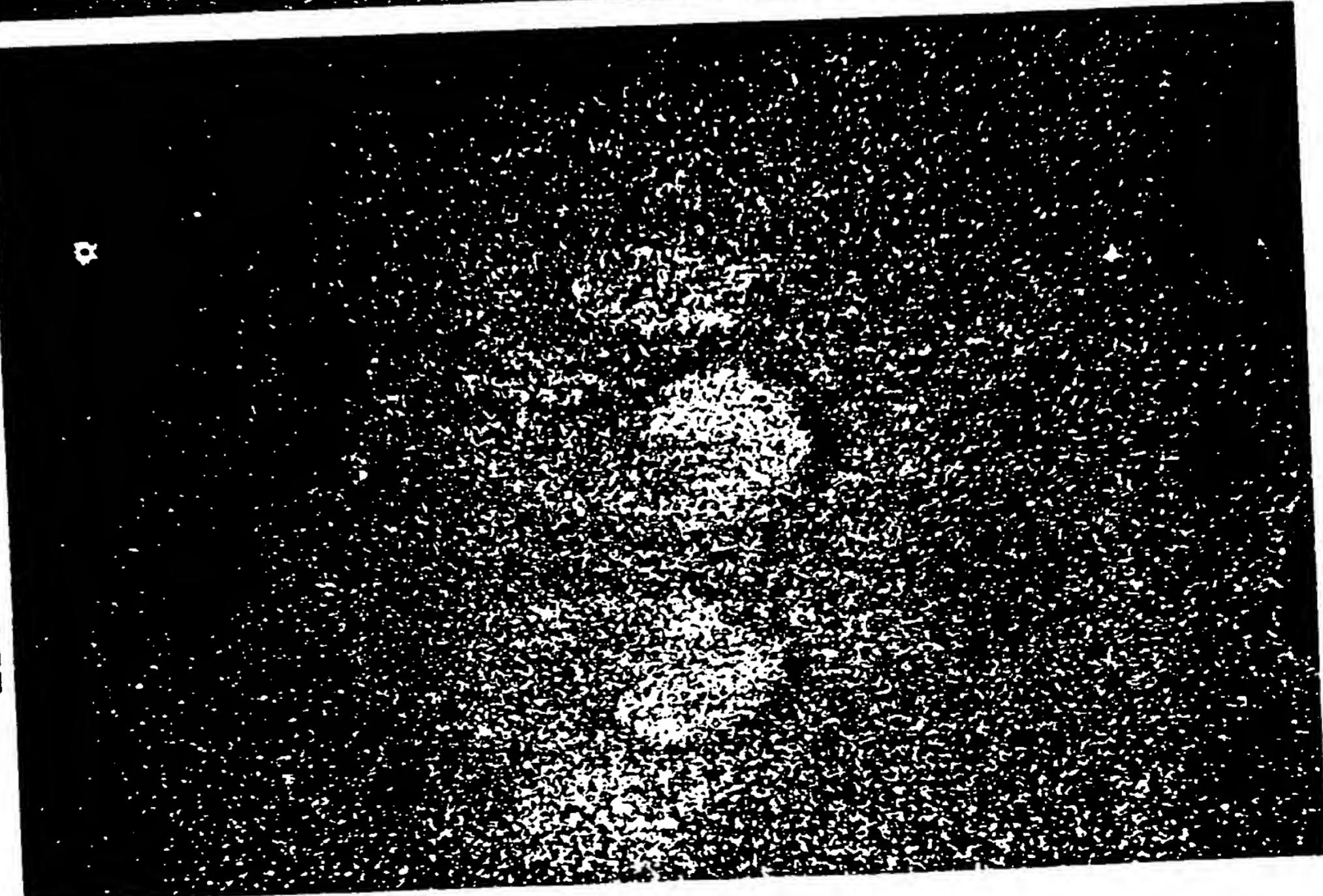
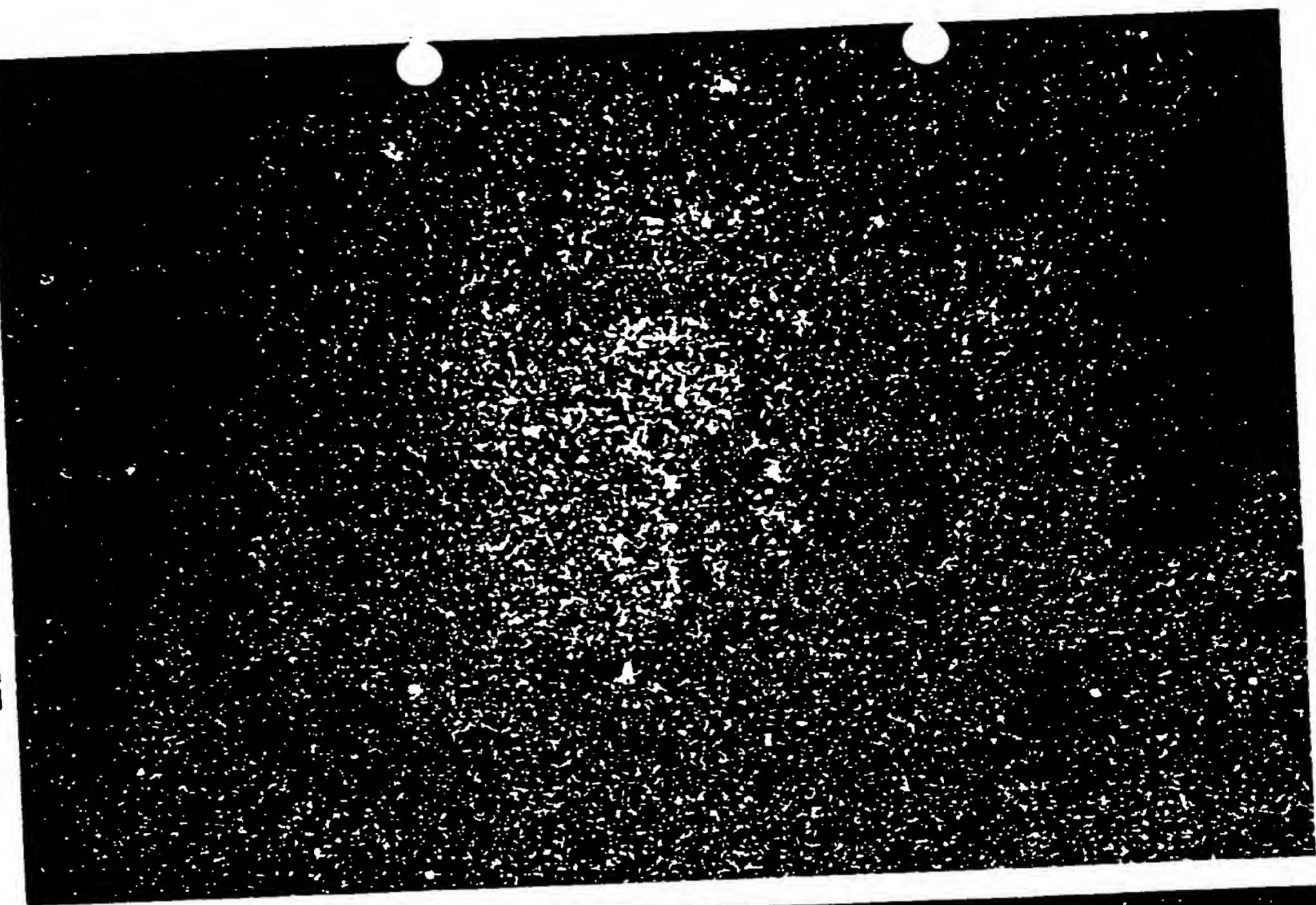


FIGURE 10B
ED5 trunk cross-section



ARIA specific probe

nARIA specific probe

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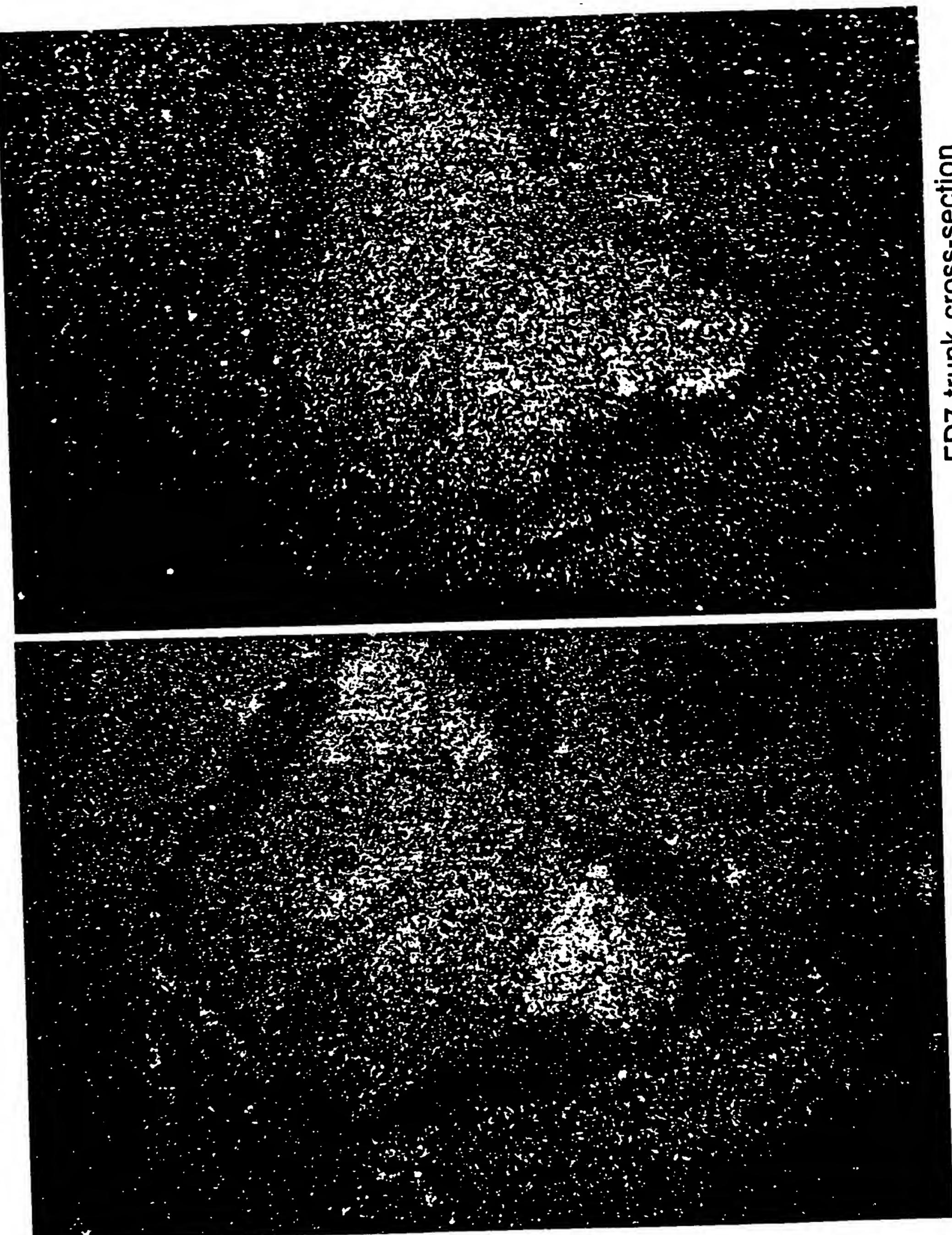


FIGURE 10D

ARIA specific probe

FIGURE 10C

nARIA specific probe

ED7 trunk cross-section

ED7 trunk cross-section

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FIGURE 11A

A. MCF-7

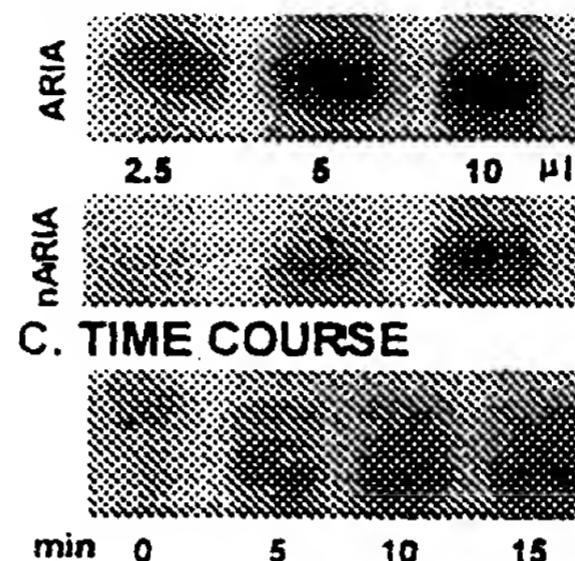


FIGURE 11B

B. LSG

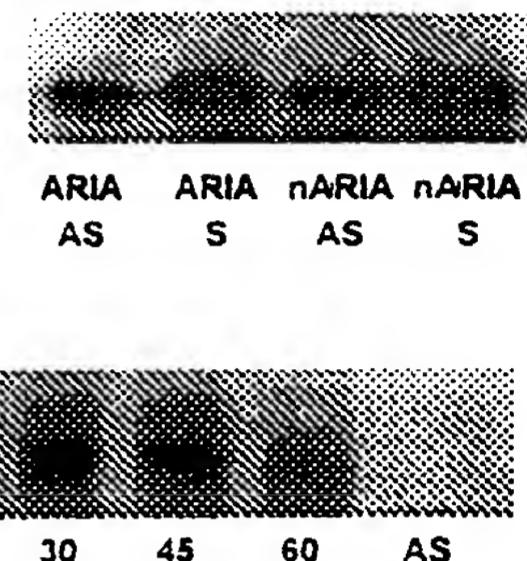


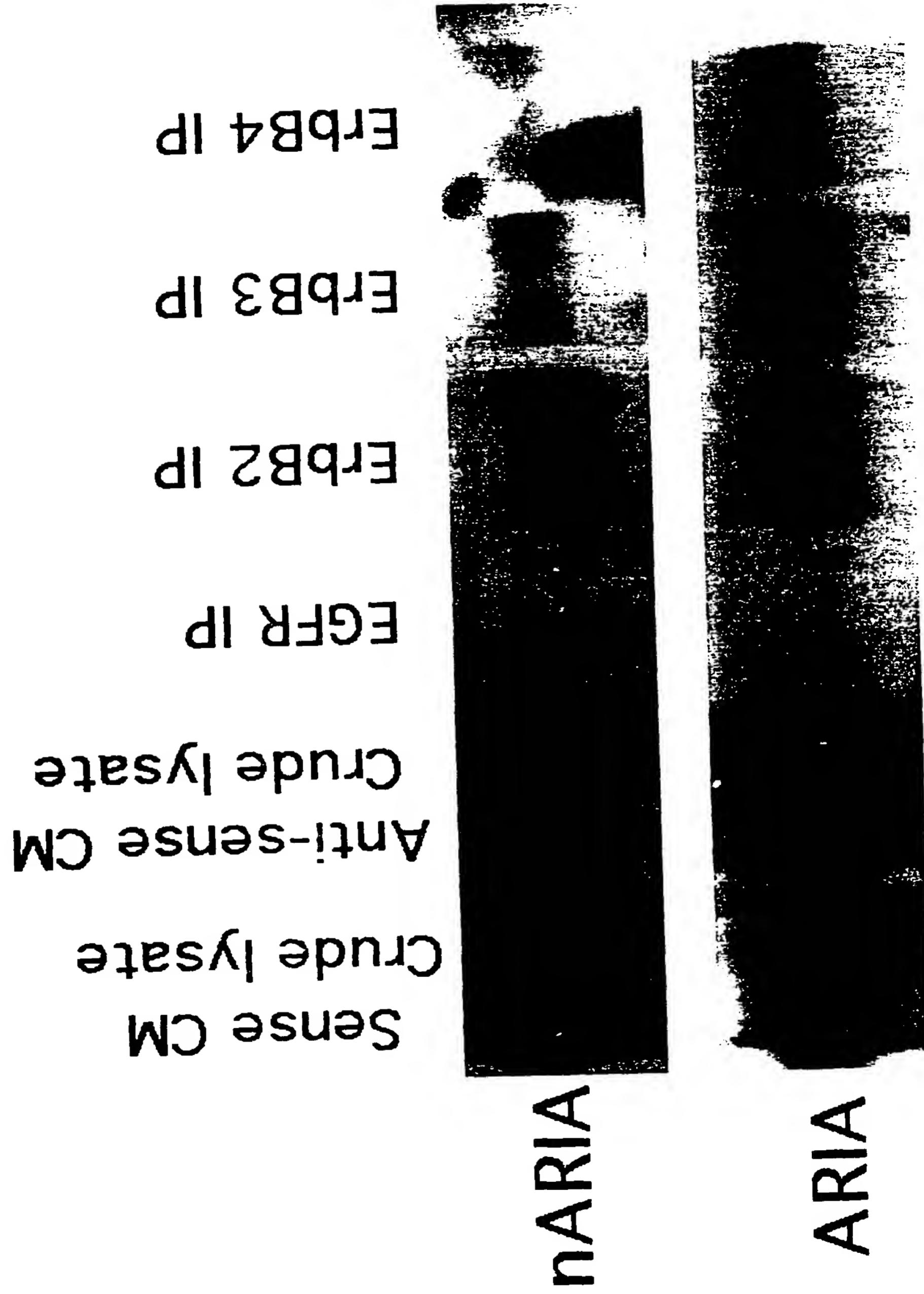
FIGURE 11C

C. TIME COURSE



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FIGURE 12



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FIGURE 13A

ED9 5% ufCEE ACh response

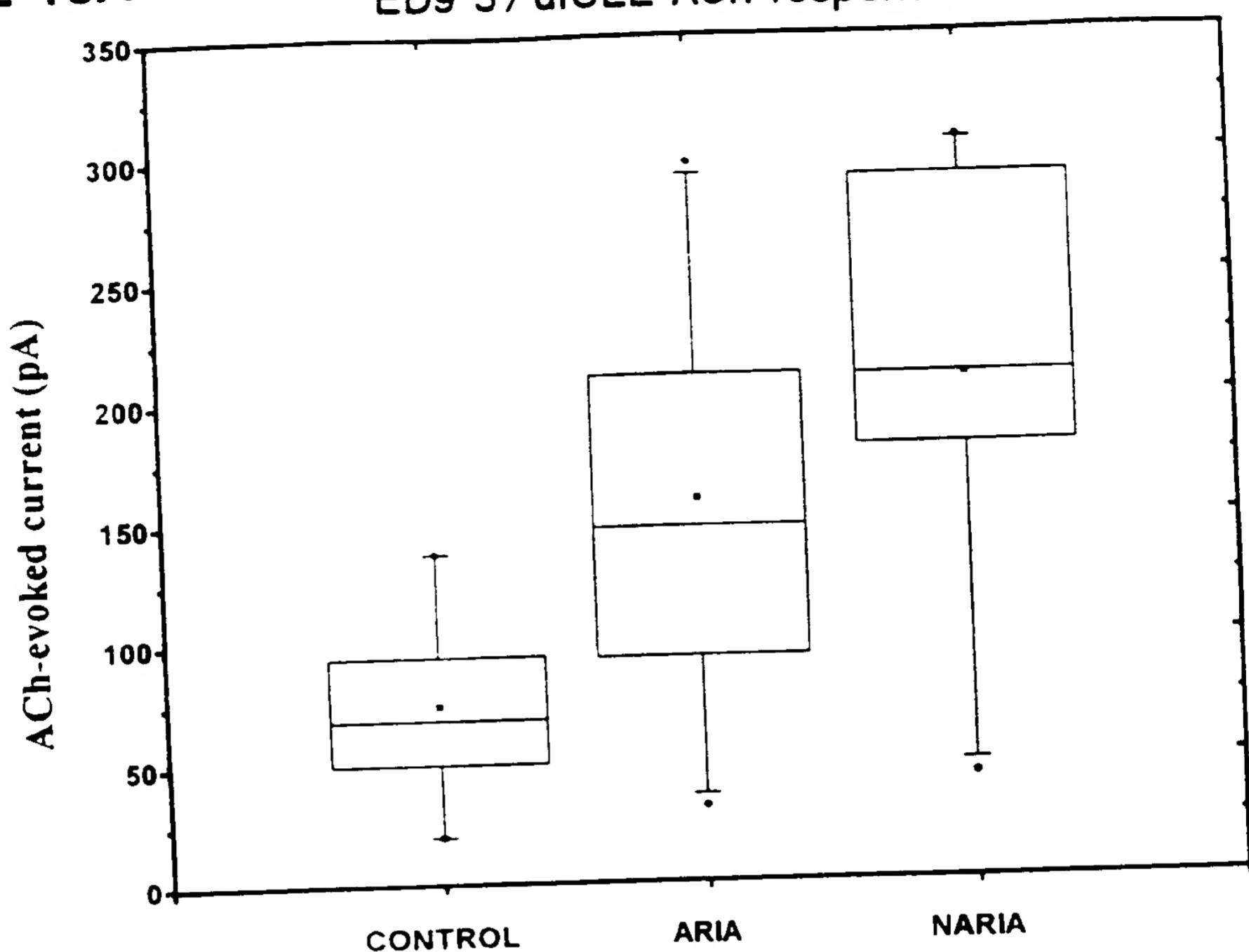
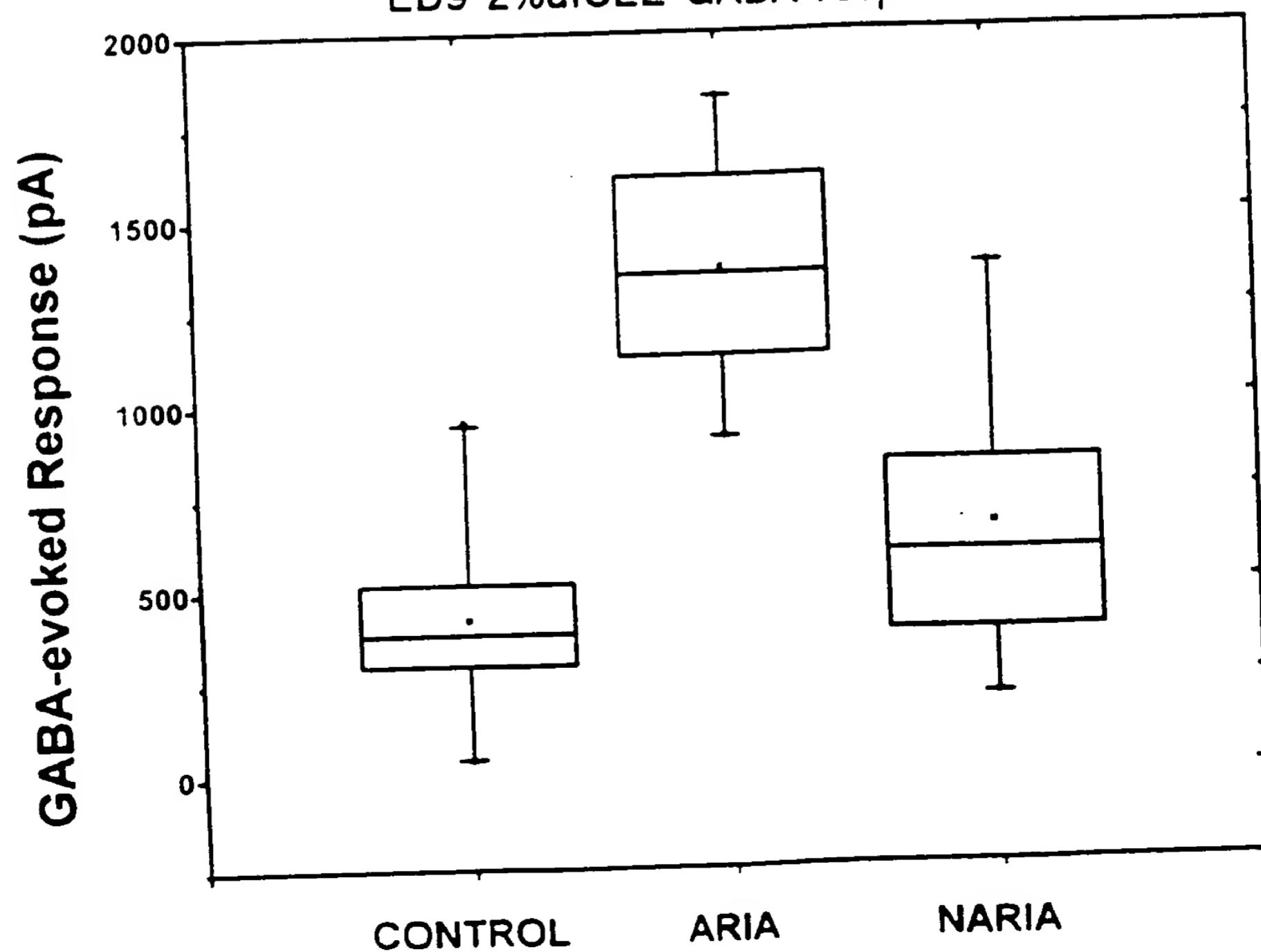


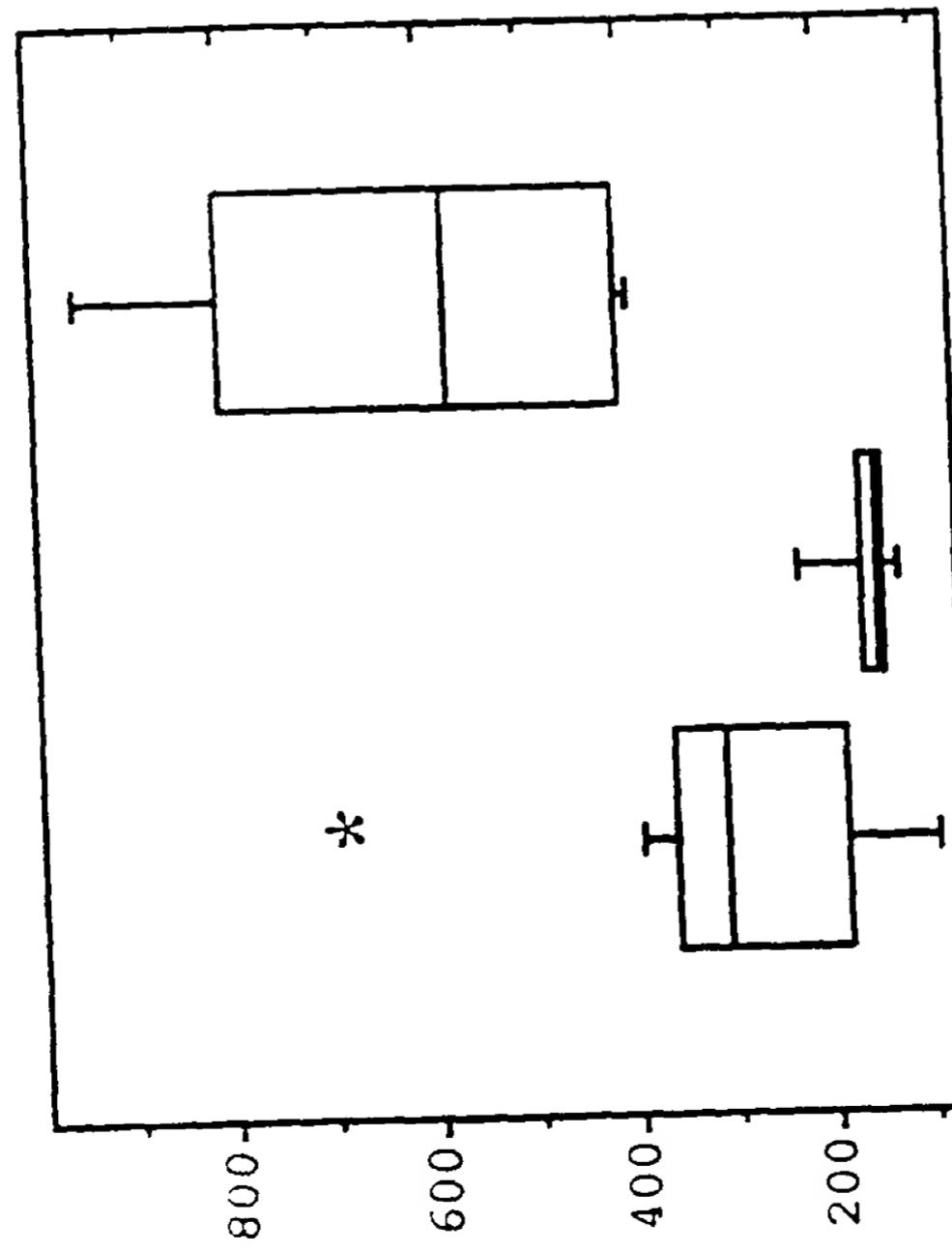
FIGURE 13B

ED9 2%ufCEE GABA response

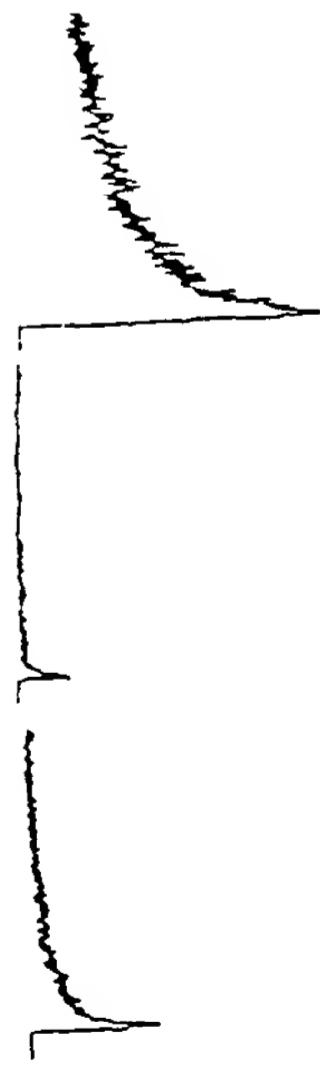


ED 11

FIGURE 13C



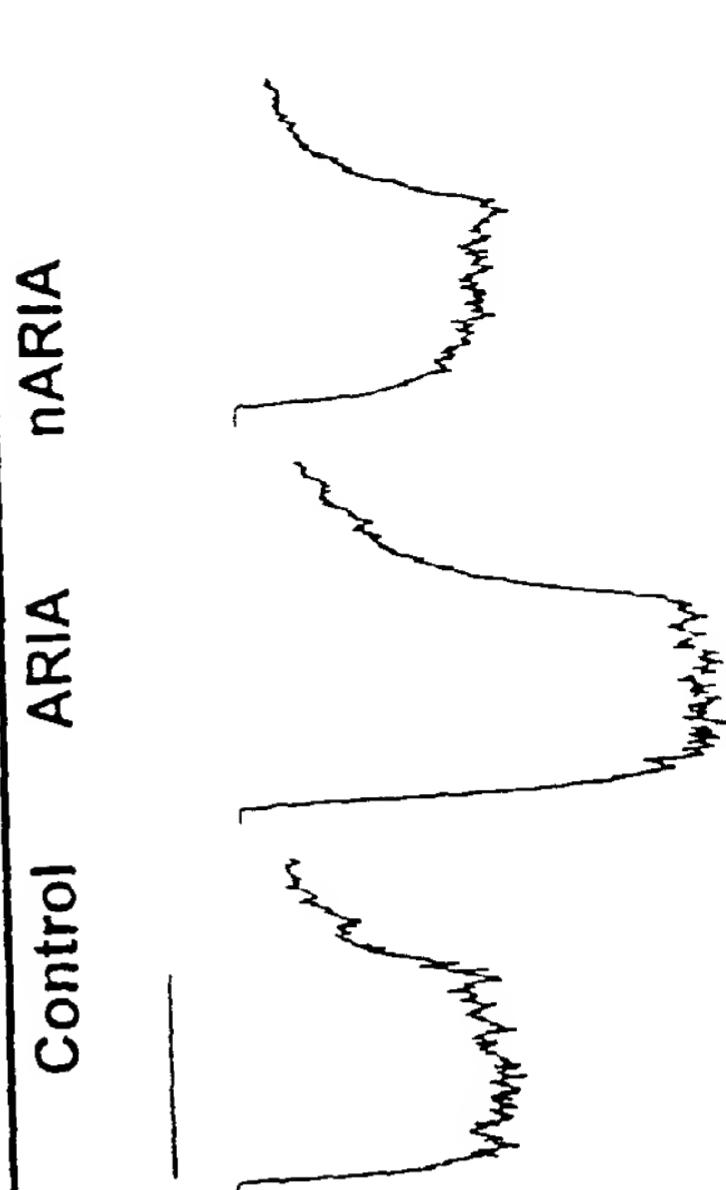
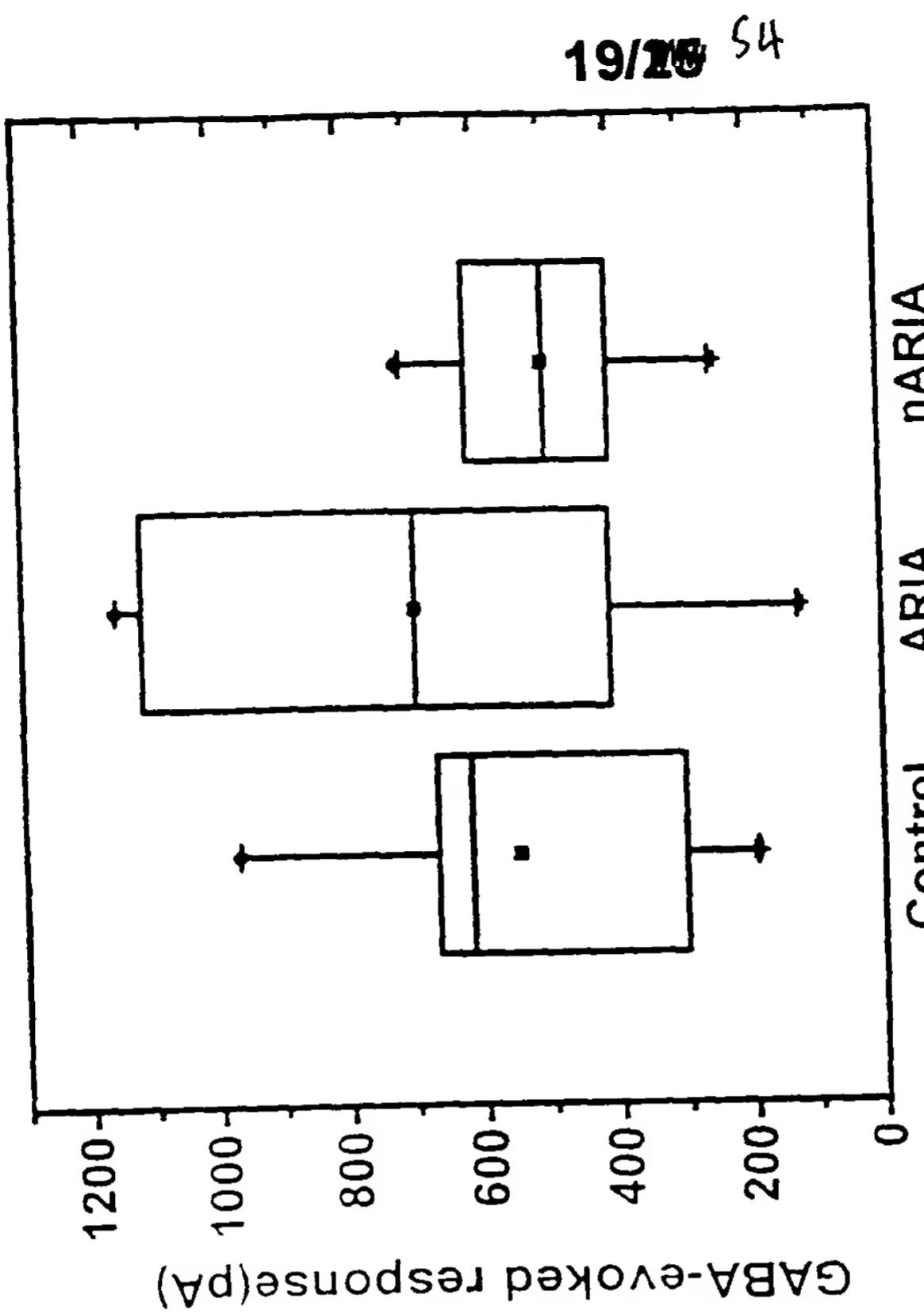
Control ARIA NARIA



Control ARIA nARIA

FIGURE 13D

ED 11



Control ARIA nARIA

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FIGURE 14A

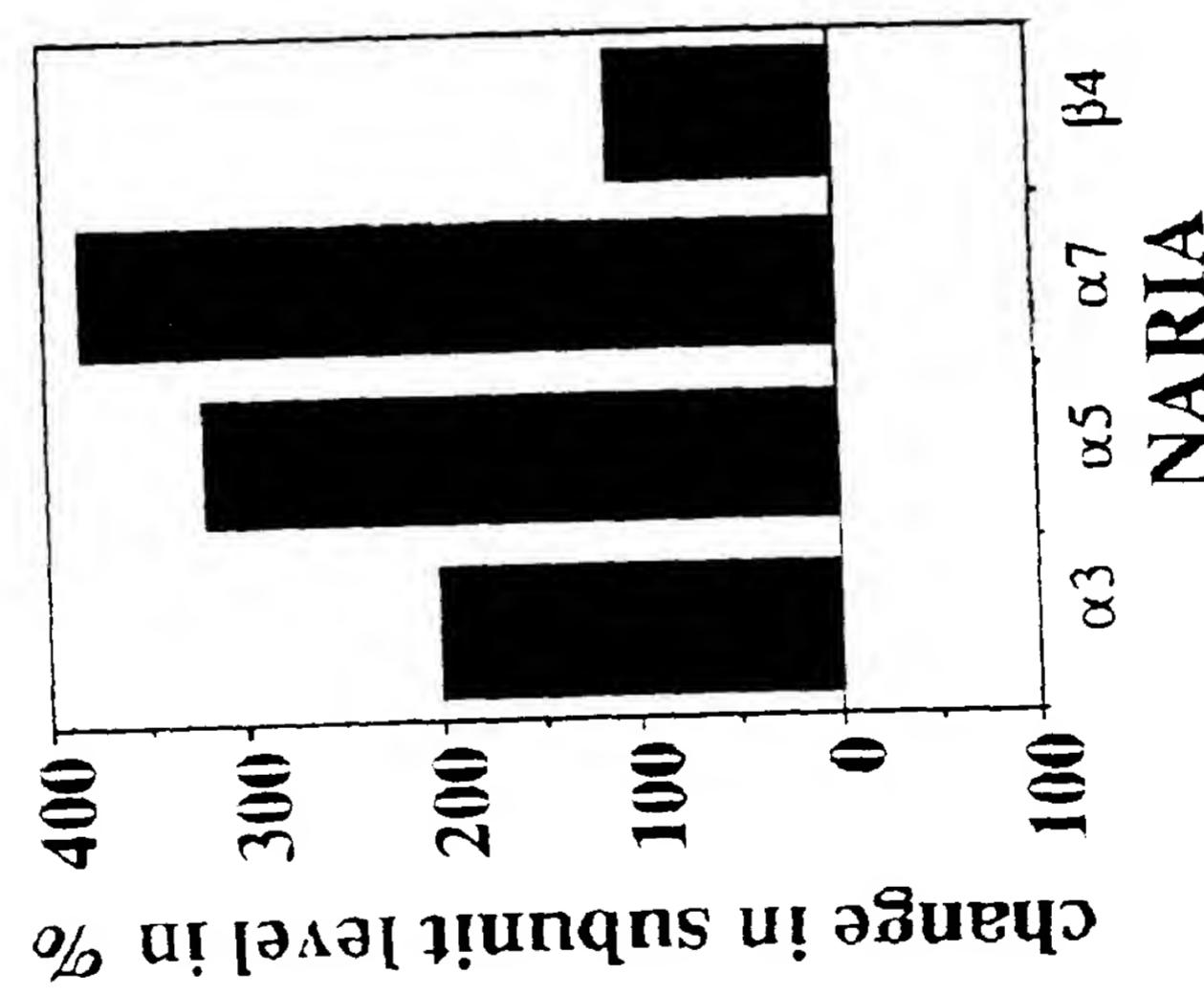
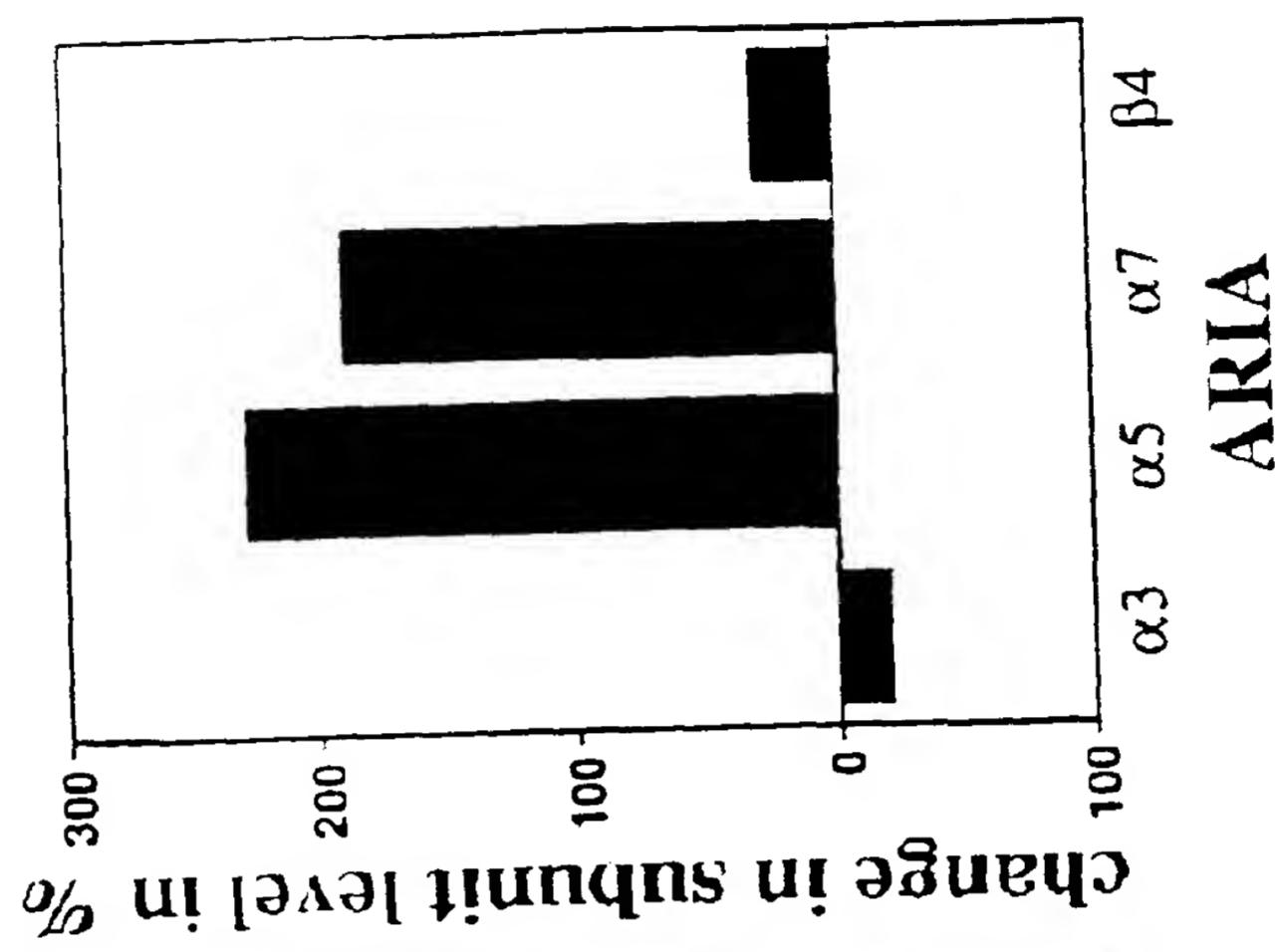


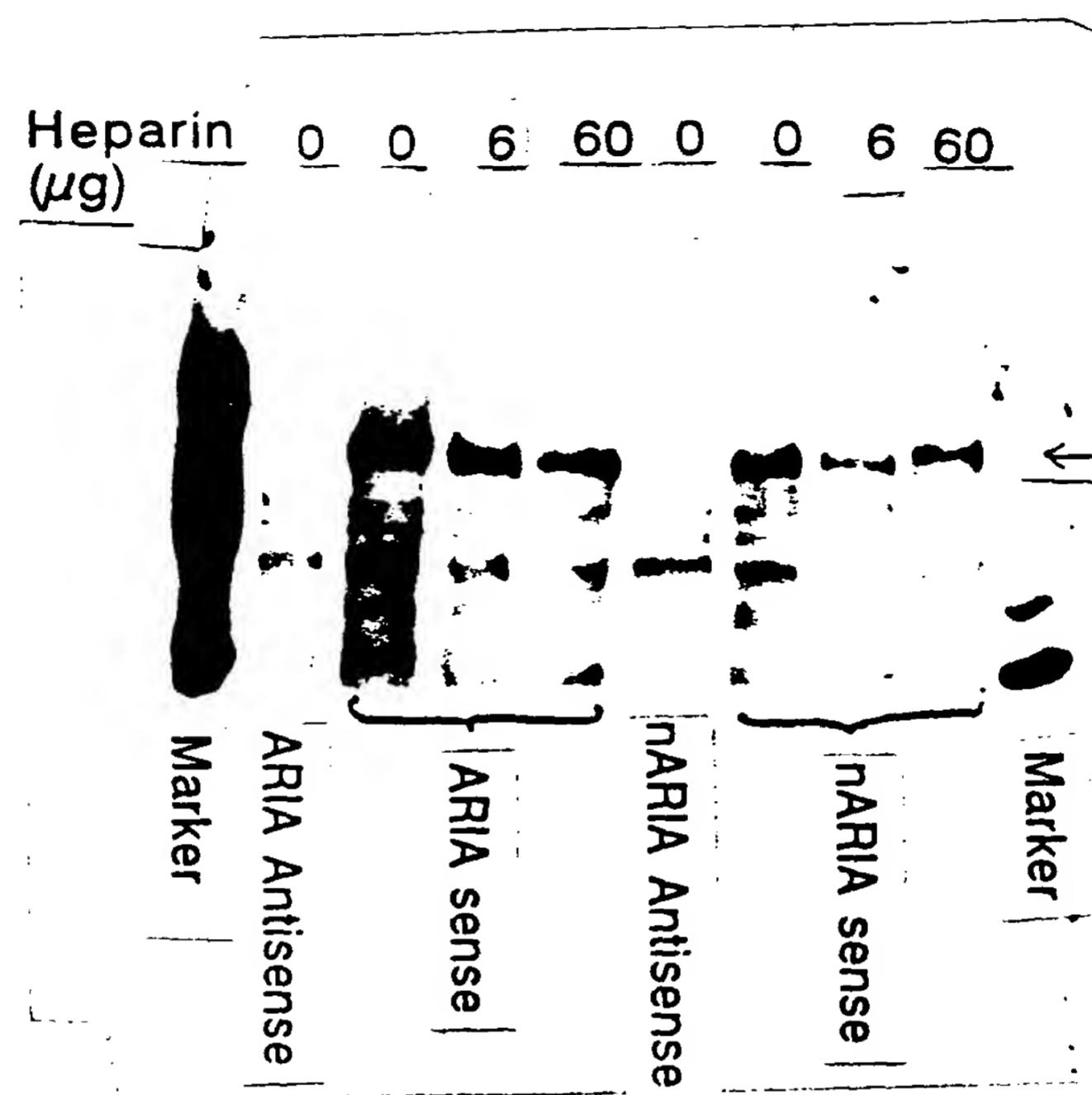
FIGURE 14B



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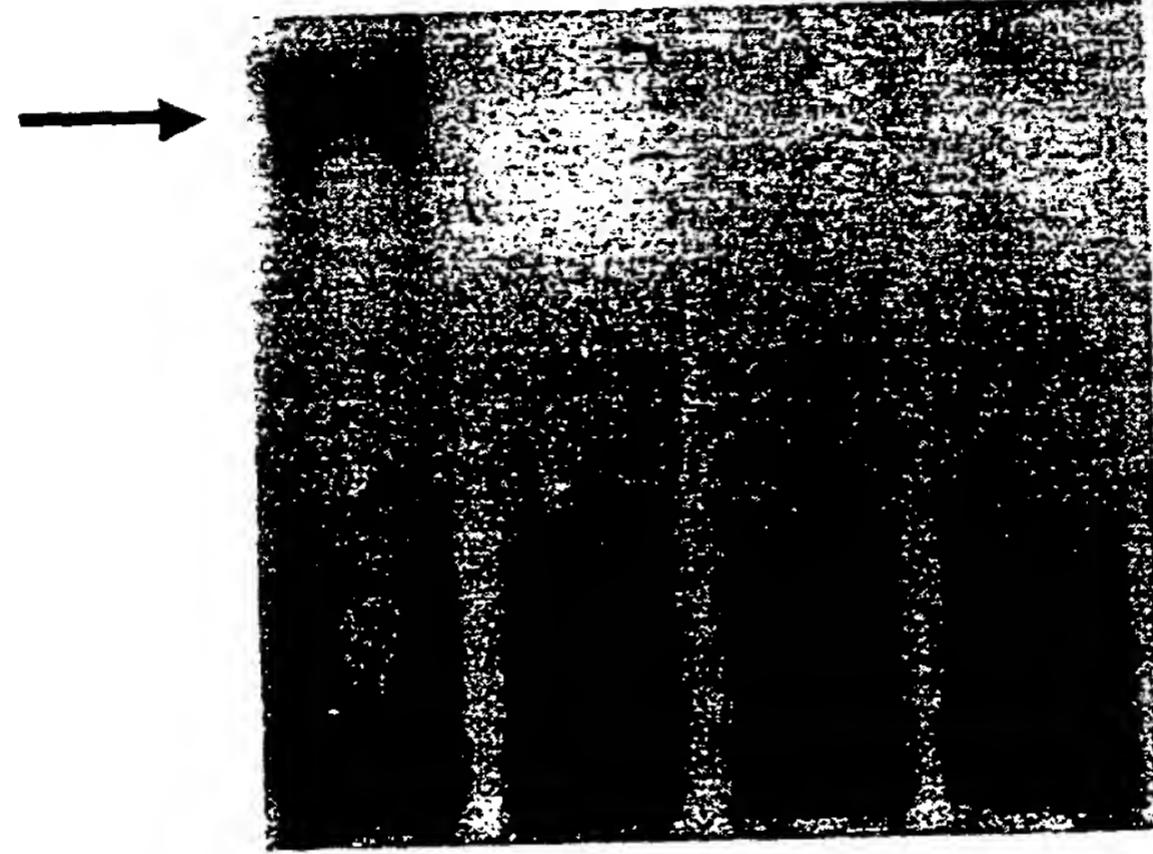
FIGURE 15



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FIG. 16

8% non-denaturing



nARIA

nARIA AS

ARIA

ARIA AS

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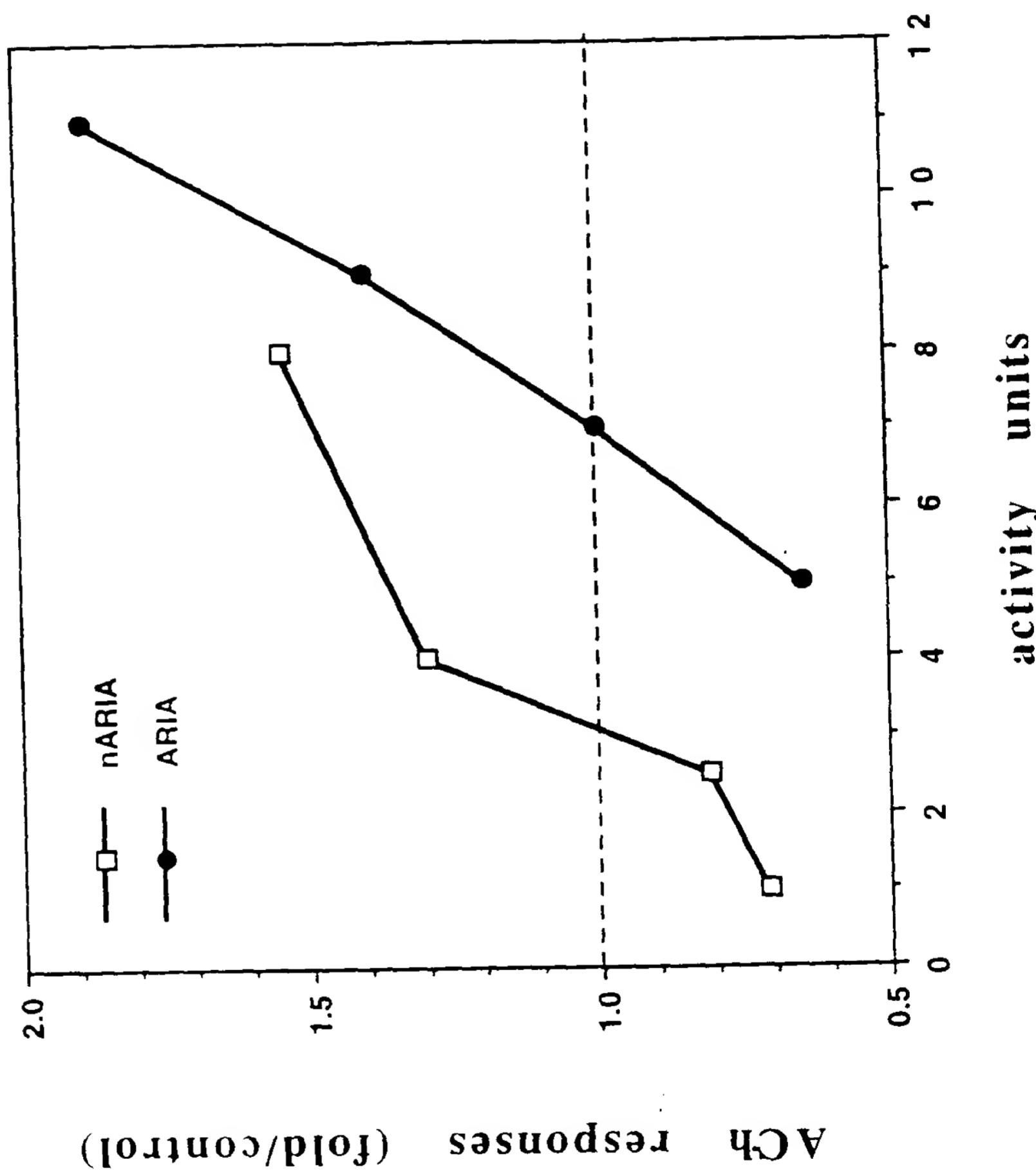
FIG. 17



Enzyme Assay - ACh E activity

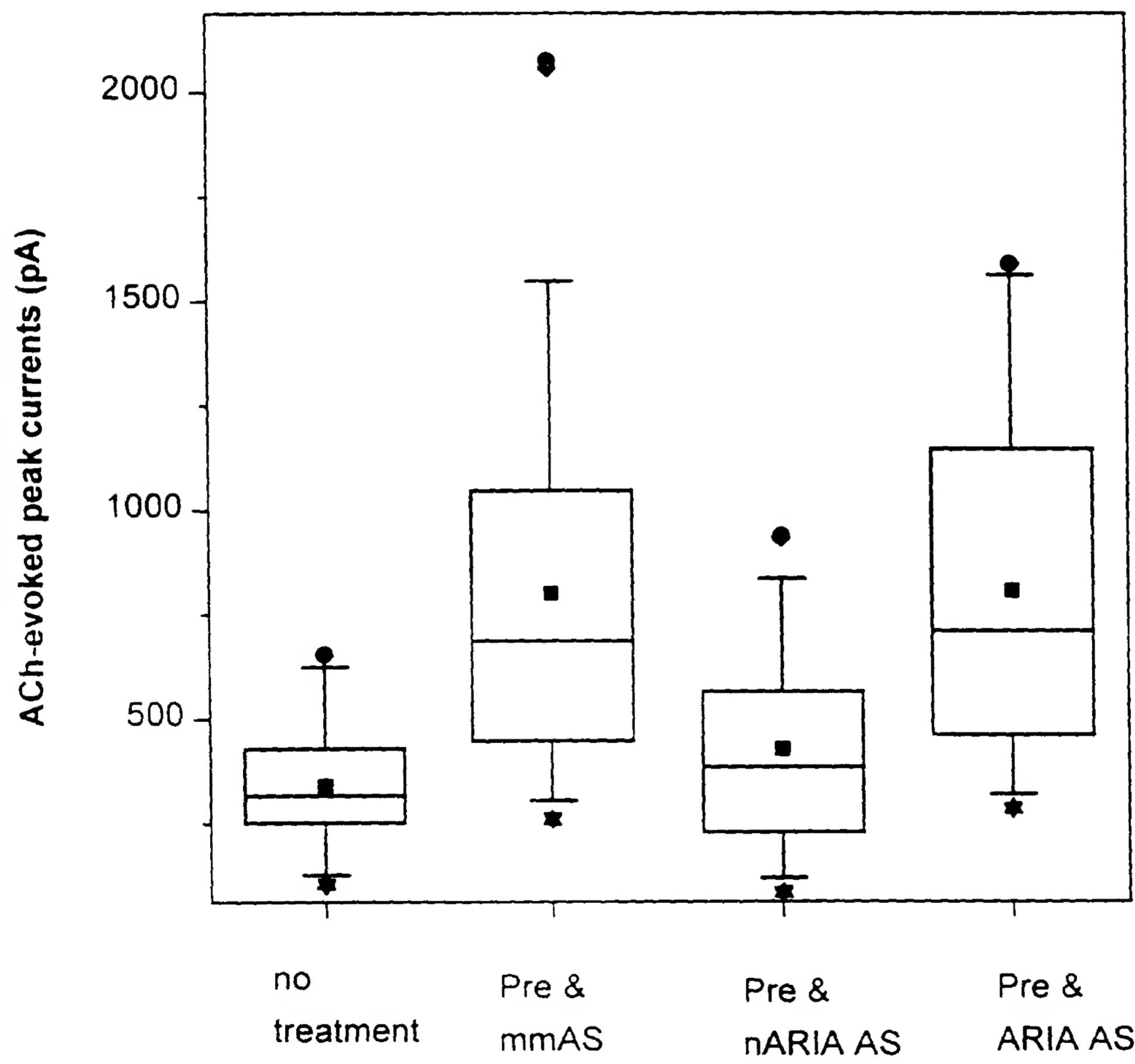
FIG. 18

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FIG. 19



no treatment=sympathetic neurons alone

'Pre'=treatment of sympathetic neurons with presynaptic input-conditioned media+various oligos
mmAS=mismatch antisense control

nARIA AS=nARIA specific antisense oligonucleotides

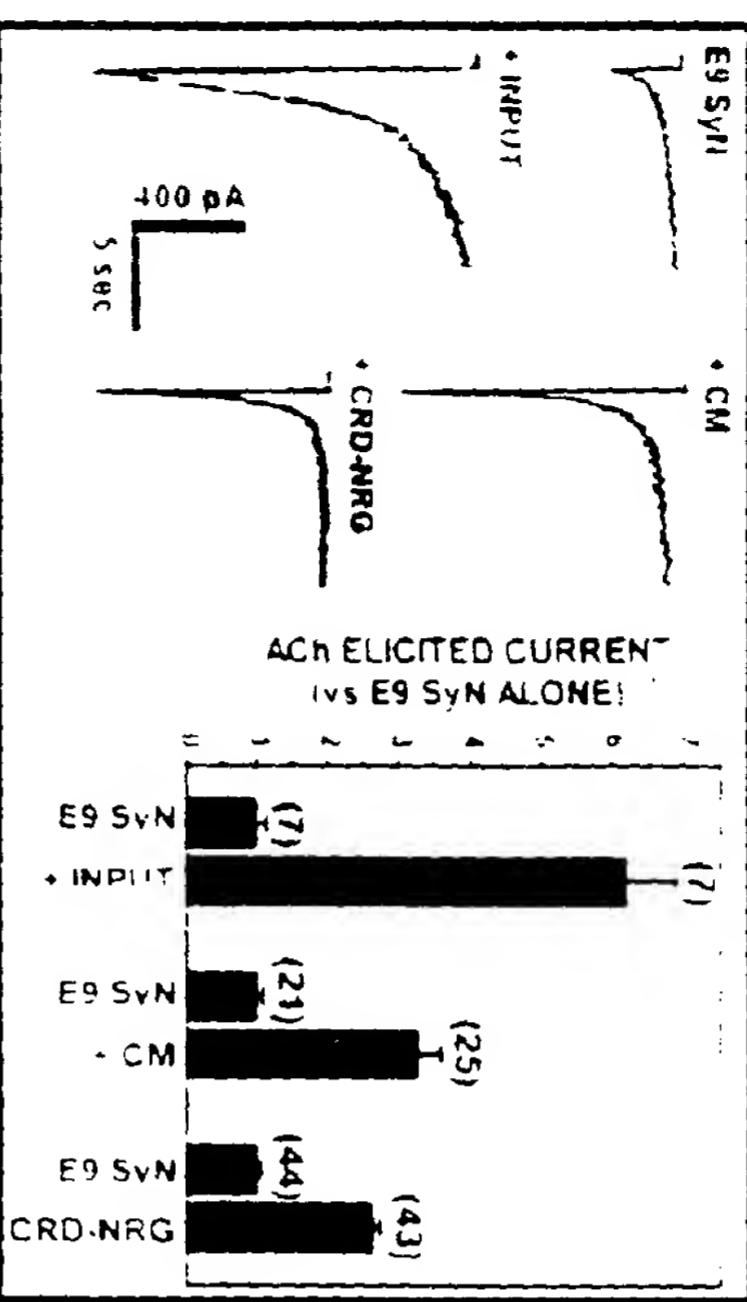
ARIA AS=ARIA specific antisense oligonucleotides

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TABLE P1-1	$\alpha 3$	$\alpha 5$	$\alpha 7$	$\beta 4$
SYMPs ALONE (set to $\equiv 1$) mRNA/SyMp (fg/100 fg std)	1.2 ± 0.2	0.4 $\pm .01$	0.2 $\pm .05$	0.4 ± 0.1
SyMps + INPUT	4.9	6.6	18	1.6
SyMps + TARGET	0.6	6.3	3.0	4.6
SYMPs+ INPUT+ TARGET	2.7	10	23	10
. <i>in vivo</i> DEVELOPMENT	2.8	11	21	12

Anterograde (Input) and Retrograde (Target) co regulation of nAChR expression utilize distinct (~ additive) mechanisms.
nAChR mRNA were assayed from synaptically naive SyNs (E9 chick) *in vitro*. Conditions indicated & presented as fold change relative to E9 SyMps ($\equiv 1$). n= (from top): 49, 51, 17, 31, 6 experiments of each condition. Single cell qPCR following electrophysiology, data corrected for amplification efficiency & actin standard (& Prog.: A2) ^RNase protection assay of E8 vs. E21, corrected for neuron number and actin standard. + heart target data: # kidney target data (see Aim 2 Progress)

A. TREATMENT with RECOMBINANT CRD-NRG MIMICS the INDUCTION of nAChRs BY INPUT & INPUT-DERIVED CM



B. FUNCTIONAL DELETION of CRD-NRG BLOCKS nAChR INDUCTION by INPUT-CM & ALTERS the NUMBER & PROFILE of SYNAPTIC nAChR CHANNELS

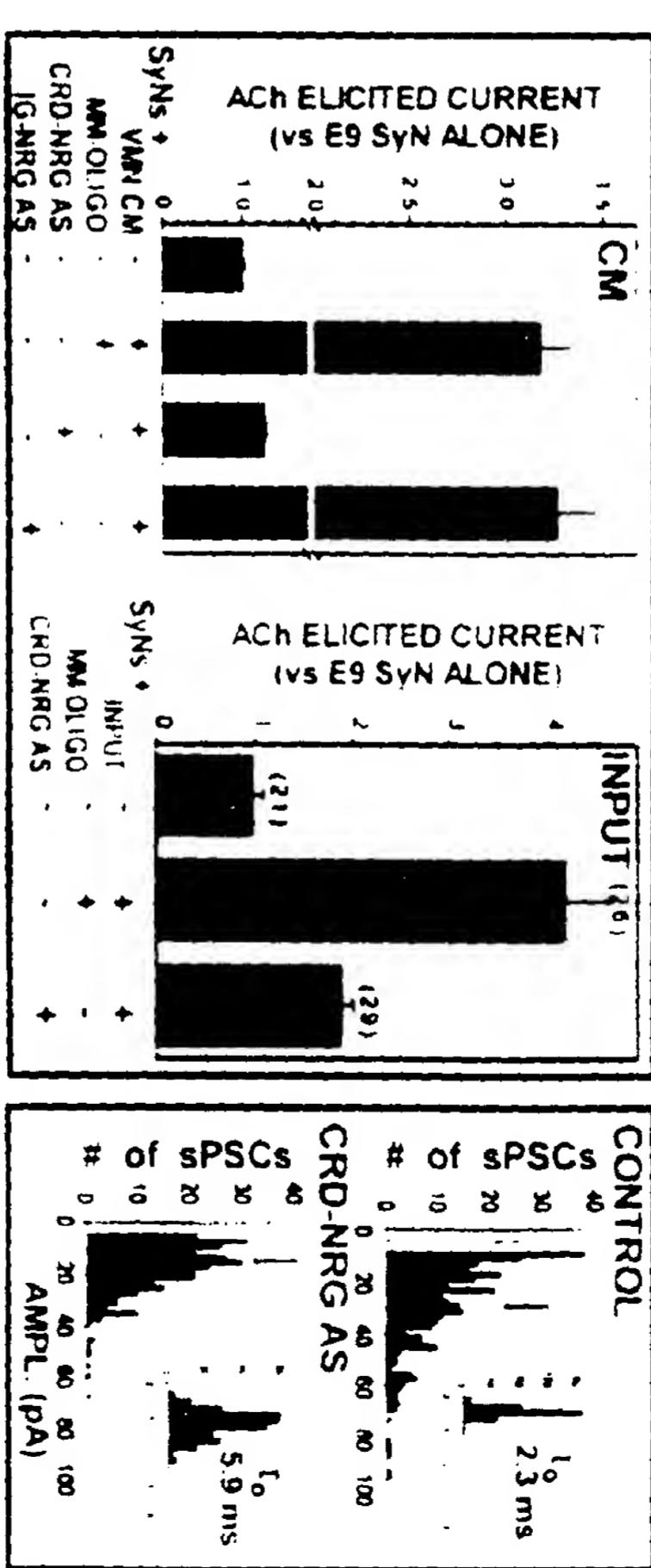


FIG. P1-1: Regulation of postsynaptic nAChRs by CRD-NRG in E9 chick sympathetic neurons.

Input-dependent induction of postsynaptic nAChRs is mimicked by CRD-NRG (**A**) and inhibited by CRD-NRG AS treatment (**B**). CRD-NRG is required for nAChR induction by input-derived soluble factors (CM) since postsynaptic nAChR induction by VMN input is strongly inhibited by CRD-NRG AS (**B**; Middle). Analysis of synaptic currents (sPSCs) at CRD-NRG "deleted" synapses reveals that this NRG isoform is required for the expression of the mature array of high β , brief α nAChR channel subtypes, normally induced by VMN input (**B**; far right). SyMPs innervated by CRD-NRG AS-treated VMN express long α , immature nAChRs, akin to those detected prior to synaptogenesis. MM = mismatch (control) oligo

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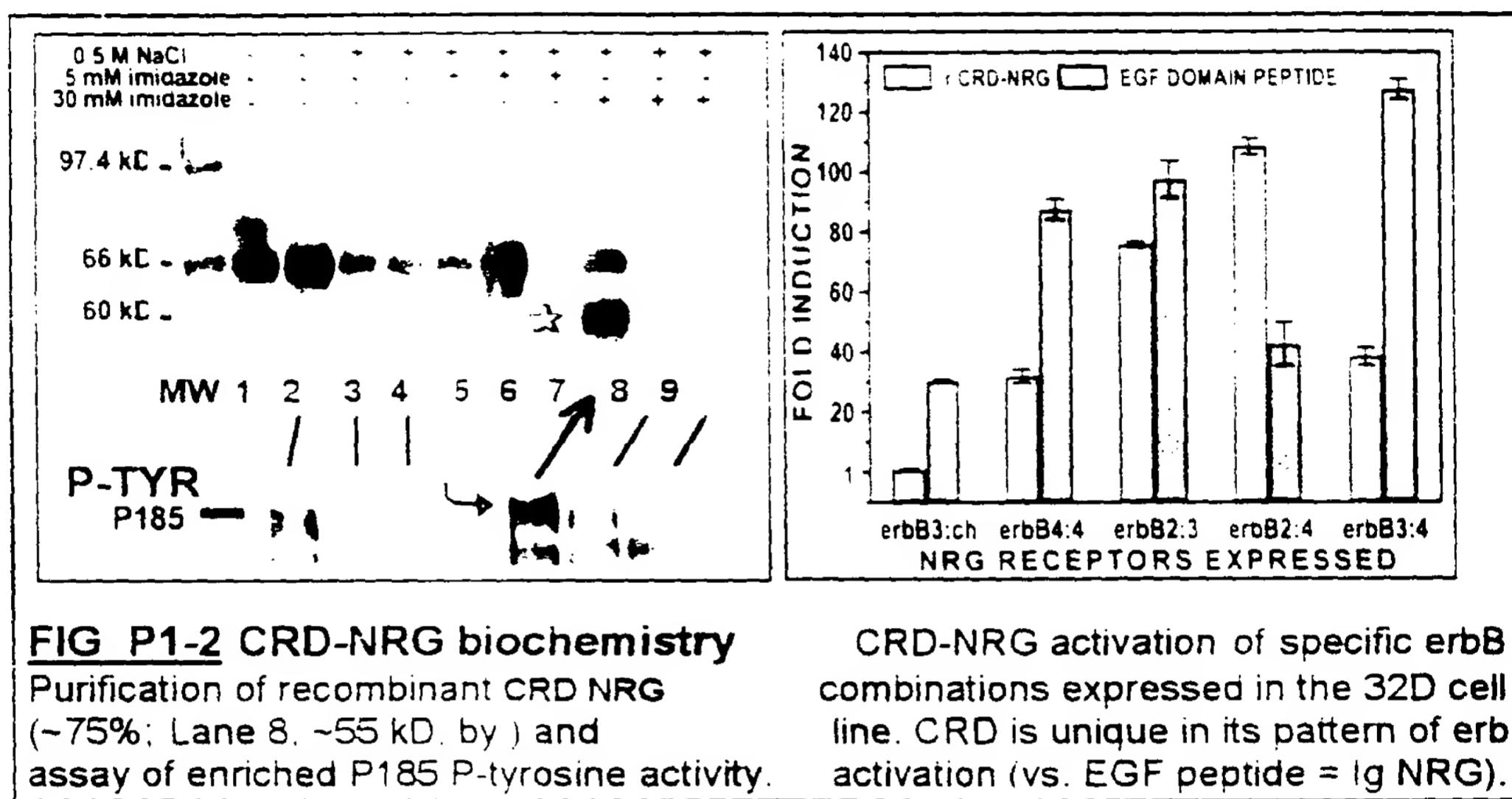


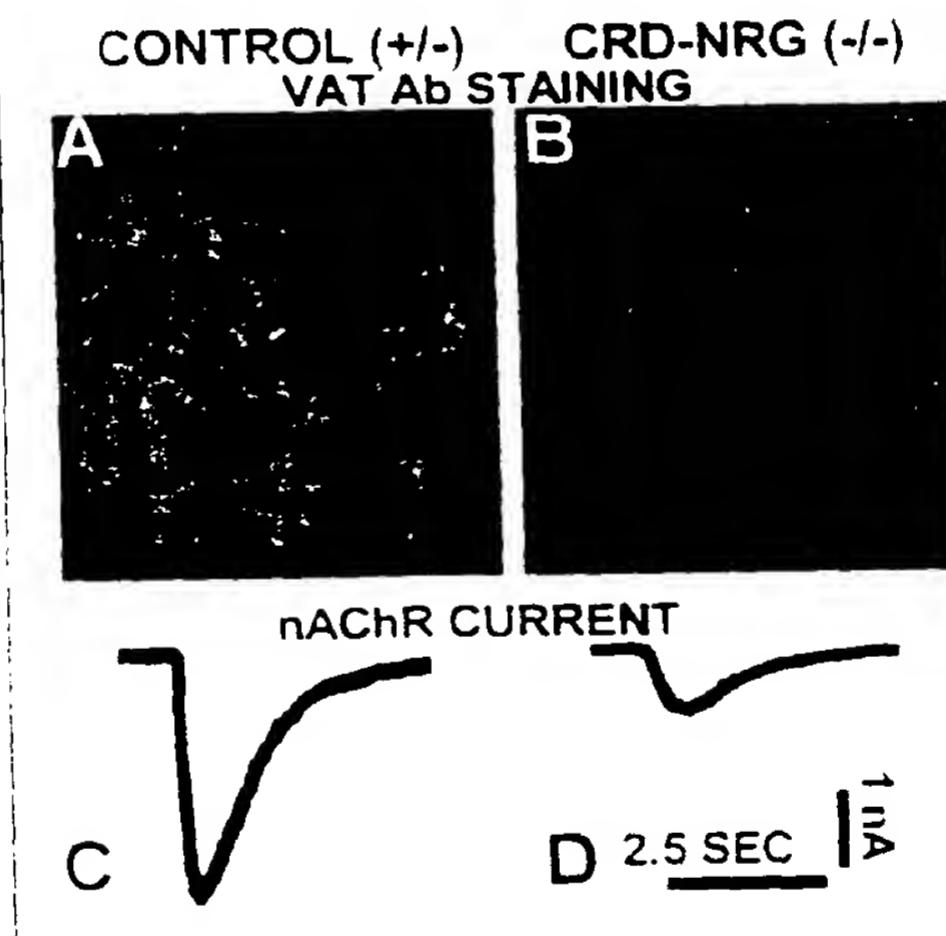
FIG P1-2 CRD-NRG biochemistry

Purification of recombinant CRD NRG (~75%; Lane 8, ~55 kD, by) and assay of enriched P185 P-tyrosine activity.

CRD-NRG activation of specific erbB combinations expressed in the 32D cell line. CRD is unique in its pattern of erb activation (vs. EGF peptide = Ig NRG).

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**Fig P1-3 VMN axons. within target
SyMp. as seen with a VAT.Ab (-A), (B)
By PO VAT+ axons and the ACh-
elicited I_p are diminished in CRD NRG
(-/-) vs control (+/-) mice. (C, D).**



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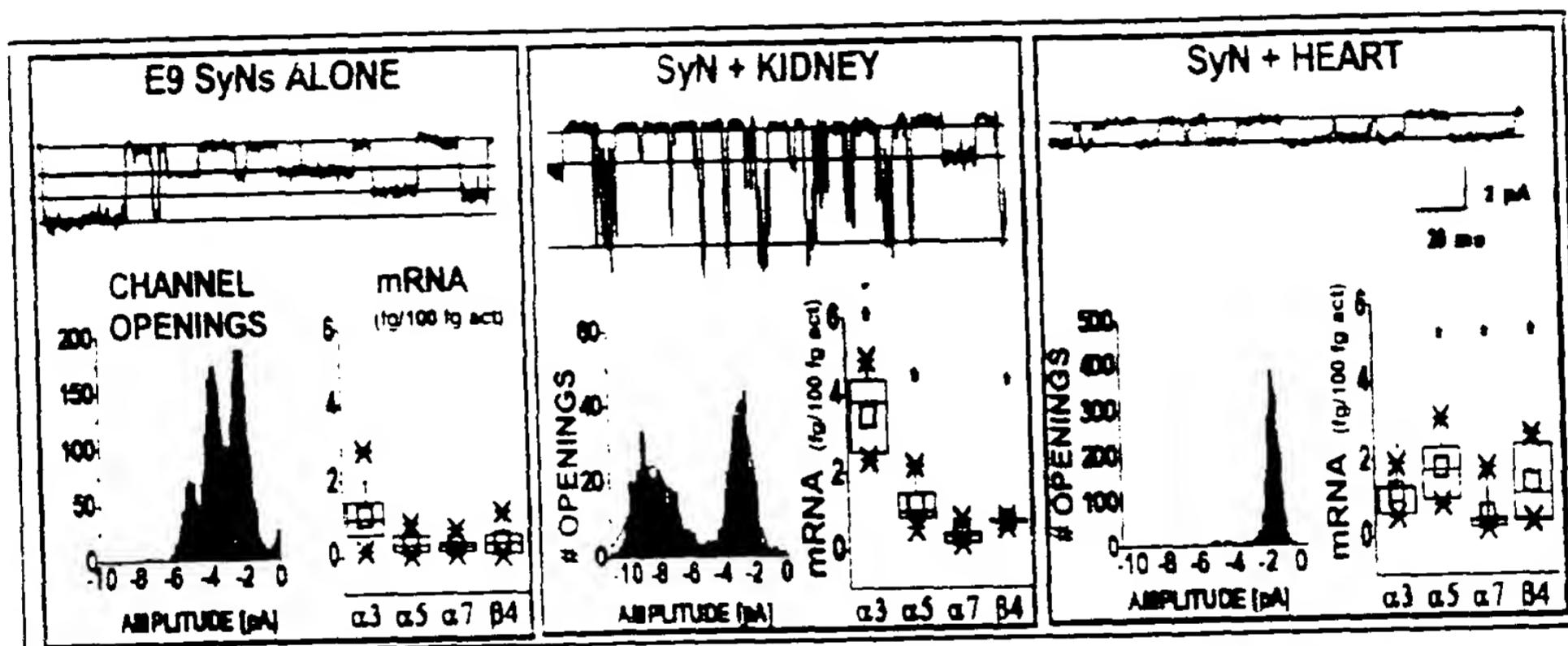
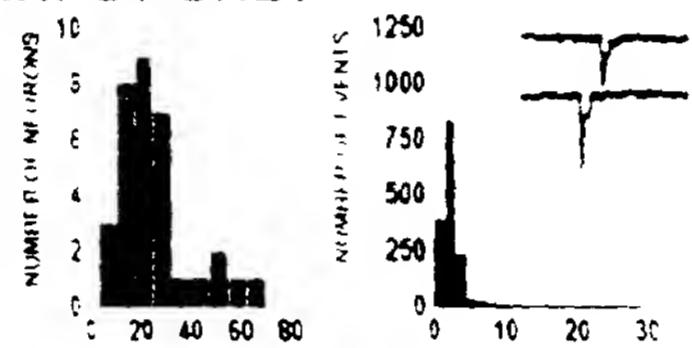


FIG. P2-1

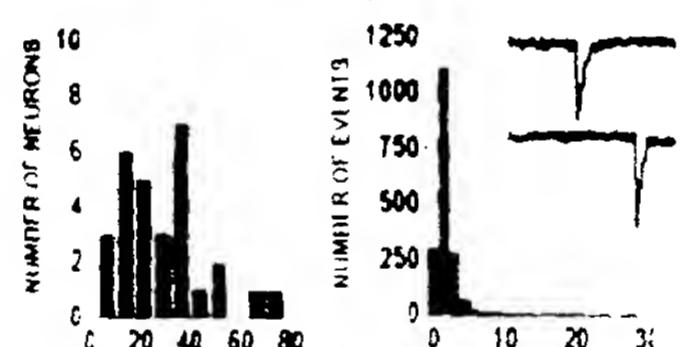
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Fig. P2-2 Target specific regulation of synaptic nAChR channels in innervated SyMp

INPUT ONLY



INPUT + KIDNEY



INPUT + HEART

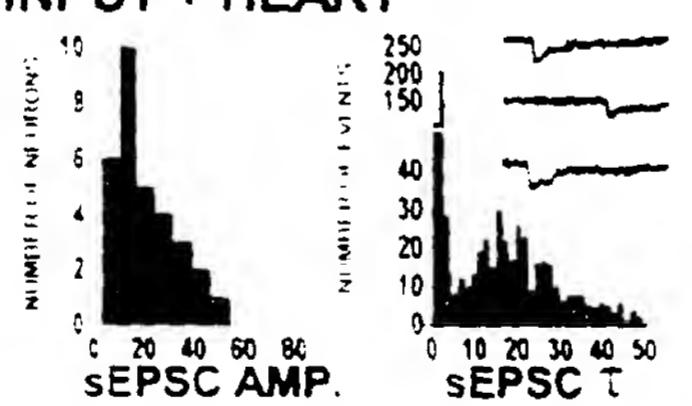


TABLE P2-1: Σ Regulation of nAChRs		($\alpha 3$) ₂ ($\beta\gamma$ x) ₃	($\alpha 3$) ₁ ($\alpha 7$) ₁ ($\beta\gamma$ x) ₃	($\alpha 3$) ₃ ($\beta\gamma$ x) ₂	($\alpha 3$) ₁ ($\alpha 5$) ₁ ($\beta\gamma$ x) ₂	($\alpha 3$) ₂ ($\alpha 5$) ₂ ($\beta\gamma$ x)	($\alpha 3$) ₁ ($\alpha 5$) ₂ ($\alpha 7$) ₁ ($\beta\gamma$ x)	$\alpha 7$
γ (pS)	13.5 ± 2	23 ± 3	28 ± 4	38 ± 6	50 ± 3	51 ± 3	66 ± 7	
P 1 (ms)	2.1 ± 5	1.1 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	3.3 ± 0.5	3.3 ± 0.4	2.5 ± 0.8	
CO ₂ (ms)	7.6 ± 1 (65%)	7.0 ± 1 (60%)	13 ± 0.9	10.8 ± 0.4 (67%)	-	16.6 ± 3.1 (39 %)		
ABUNDANCE								
Early develop.	++++	-	++	-	+	-	-	
Intermediate	+++	+	++	+++	+++	+++	+	
Late develop.	-	++	-	+++	+++	+++	++	
INDUCED by...								
Input	-	-	-	-	++	++	-	
Contact with kidney	-	-	-	+	+++	+++	++	
Contact with heart	++++	++	+	-	+++	+++	+++	
PHARMACOLOGY		the number of +'s represents the relative apparent affinity for ACh (where > +'s indicates > apparent affinity)						
ACh (rel. K _{app})	++++	+++	+++	++	++	++	+	+
Cytisine ¹	+	-	+	-	+	-	-	ND
n-BgTx sensitivity	+	+	+	+	+	+	+	+
α -BgTx sensitivity	-	+	-	-	-	-	-	ND
MLA sensitivity ²	-	-	-	+	-	+	+	+
DELETED by AS to :	$\alpha 3$	$\alpha 3, \alpha 7$	$\alpha 3$	$\alpha 3, \alpha 5, \alpha 7$	$\alpha 3, \alpha 5$	$\alpha 3, \alpha 5, \alpha 7$	$\alpha 7, ND$	

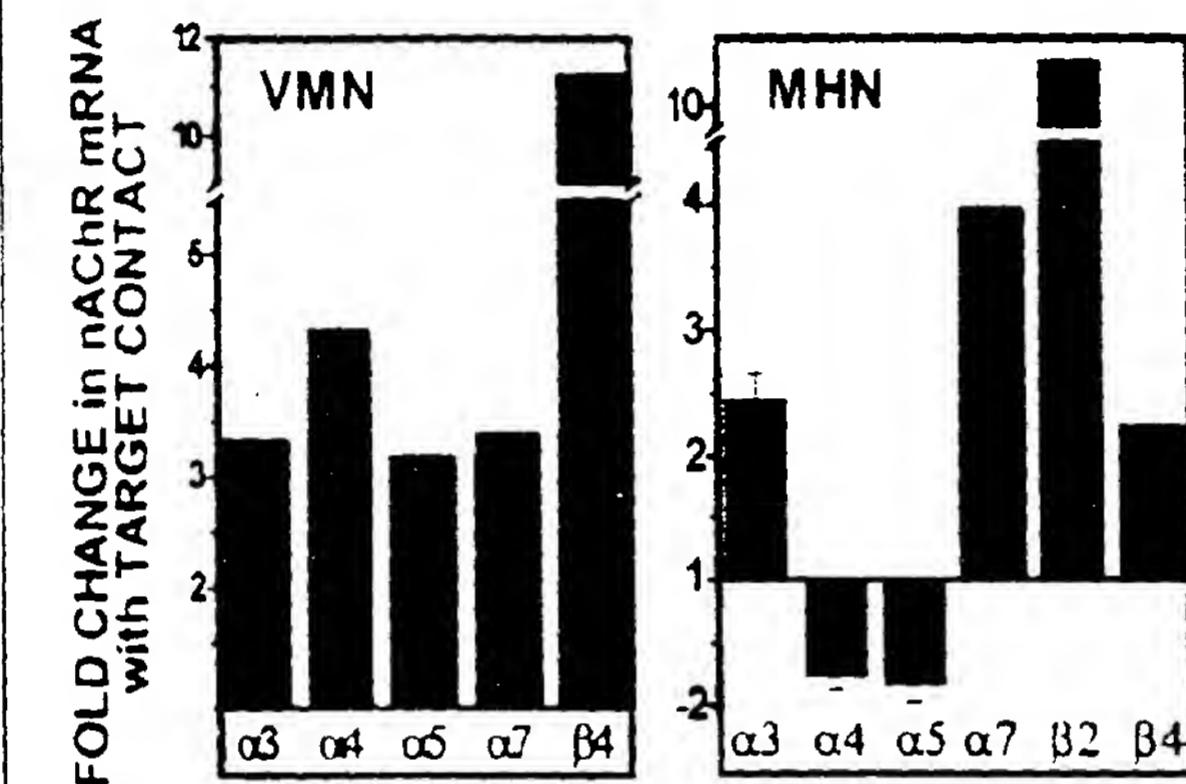
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You have made it to AIM 1 FIGS AND TABLES

	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 7$	$\beta 2$	$\beta 4$	
Visc. Motor							
E18 vs. P0, mouse	↑	↑	-	↑	↑	↑	ND
E9 vs. E18, chick	ND	↑↑	↑	↑	↓	↑	
Med. Habenula							
E16 vs. P0, mouse	↑	↑	↑	↑	↑↑	↑↑	ND
E11 vs. E17 chick	↓	-	↑	↑↑	↑↑	↑↑	↑↑
qPCR assay of chick tissue extracts: mouse data from "side-by-side" <i>in situ</i> assays (^{FN1} , Methods).							
ND: not determined: - no change or low signal.							

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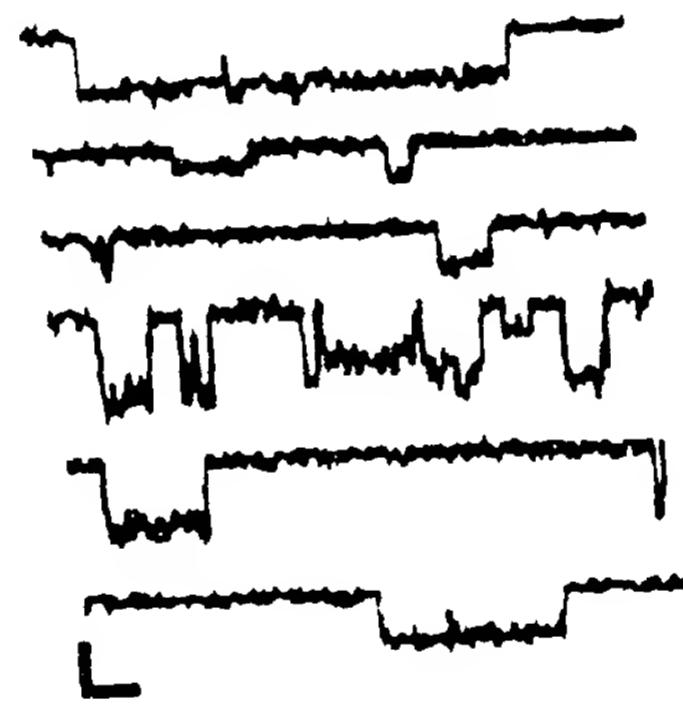
FIG A1-1: The profile of nAChR subunit gene expression in presynaptic VMN and MHN neurons is strongly regulated by interaction with neuronal targets *in vitro*.



nAChR subunit mRNA levels were assayed by qPCR of chick neurons *in vitro* - synaptic partners.. The levels of subunit mRNA in "synaptically naive" neurons is set to 1.

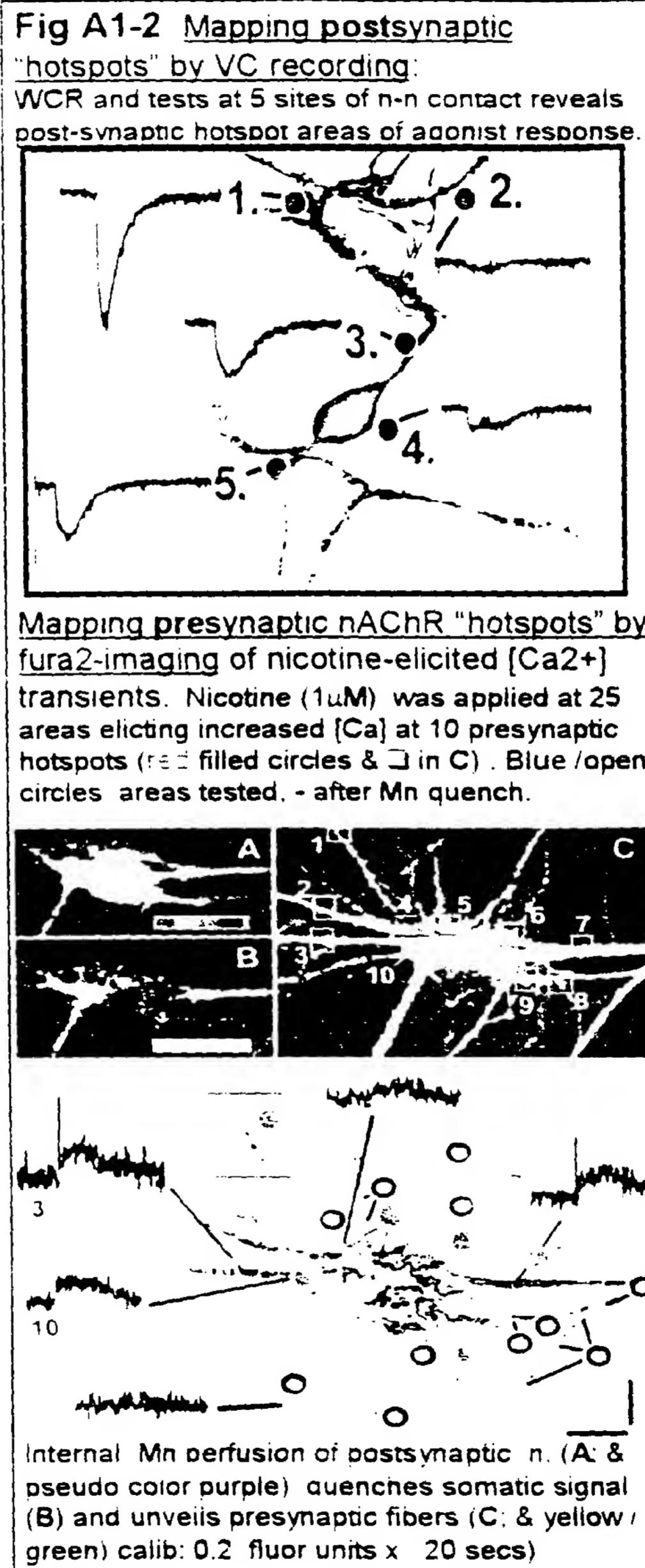
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Sample recordings of
mouse nAChRs



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1996-02-06



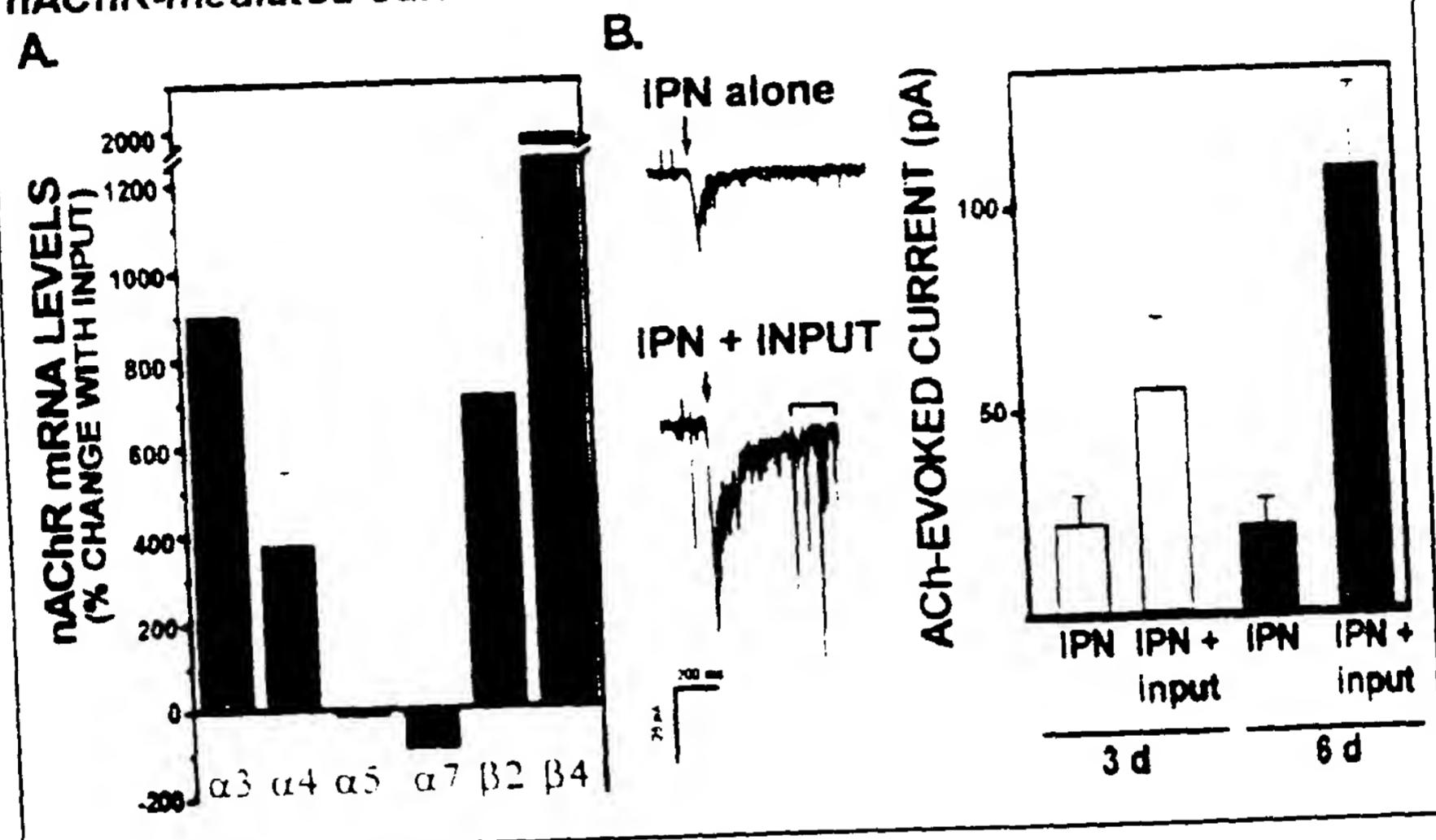
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TABLE A1-2	AChE Fibers	α_3	α_4	α_5	α_7	β_2
IPN						
E16	+	-	-	+	-	++
E18/P0	+++	+/-	+	++	+/-	++
P7	+++	++	++	+++	++	+
AMYG						
E16	-	-	-	-	-	-
E18/P0	+ (gc)	+/-	++	+/-	+	+//+
P7	+++	ND	+++	+	+++	++

"Amygdala" refers to 2 major cholinceptive subregions: the basoventral nucleus (BLA), and the Nucleus of the lateral olfactory tract nuclei (NLLOT). (gc)= growth cone tipped AChE + fibers. ND= not determined.

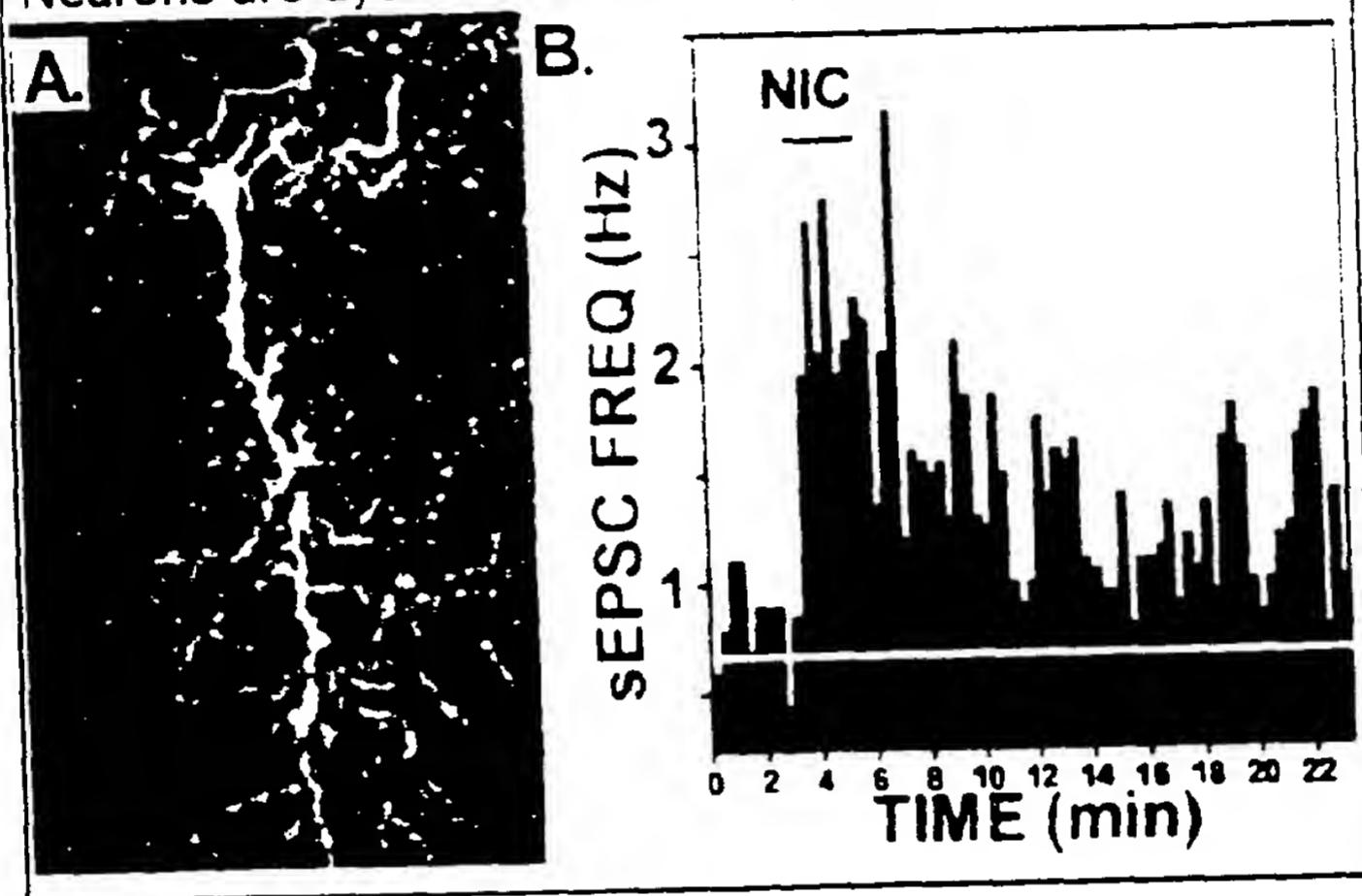
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Fig A1-3: *In vitro* innervation of IPN by MHN alters the profile of nAChR subunits expressed and increases the magnitude of nAChR-mediated currents



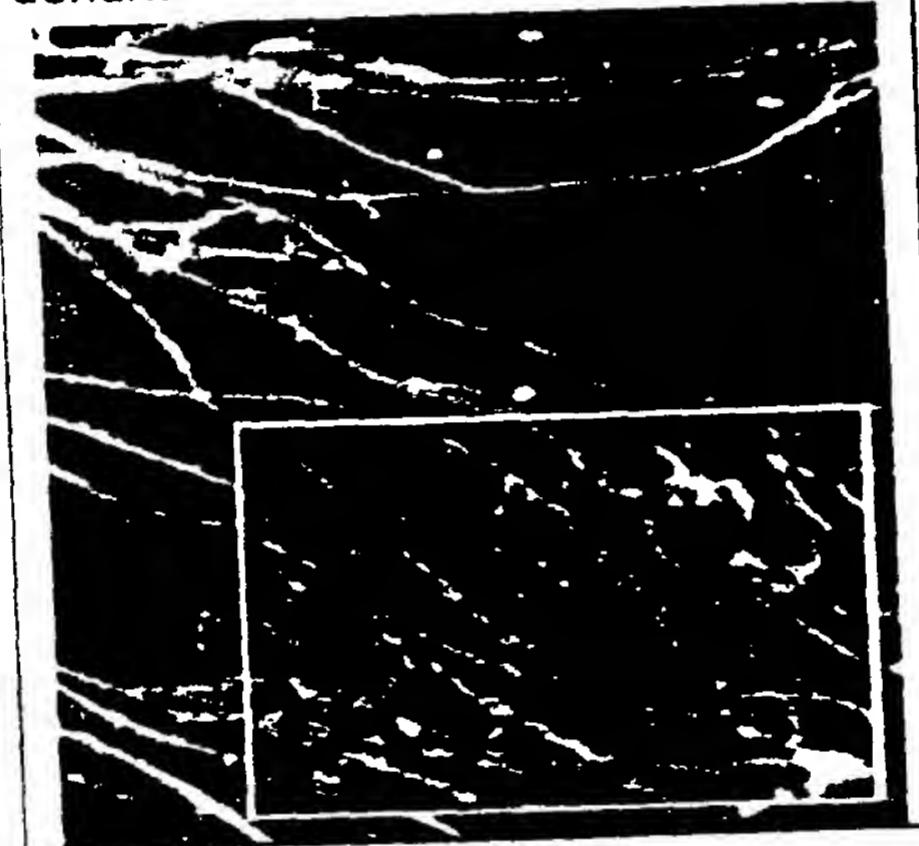
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Fig A1-5: Nicotine induces robust synaptic facilitation: slice-patch recording from P0 mouse IPN (**B**). Neurons are dye filled for subsequent re-location (**A**)



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Fig A1-4: Cholinergic fibers exit mouse MHN micro-explants (VAT+; red). Contacts with IPN MAP + dendrites are common (green).



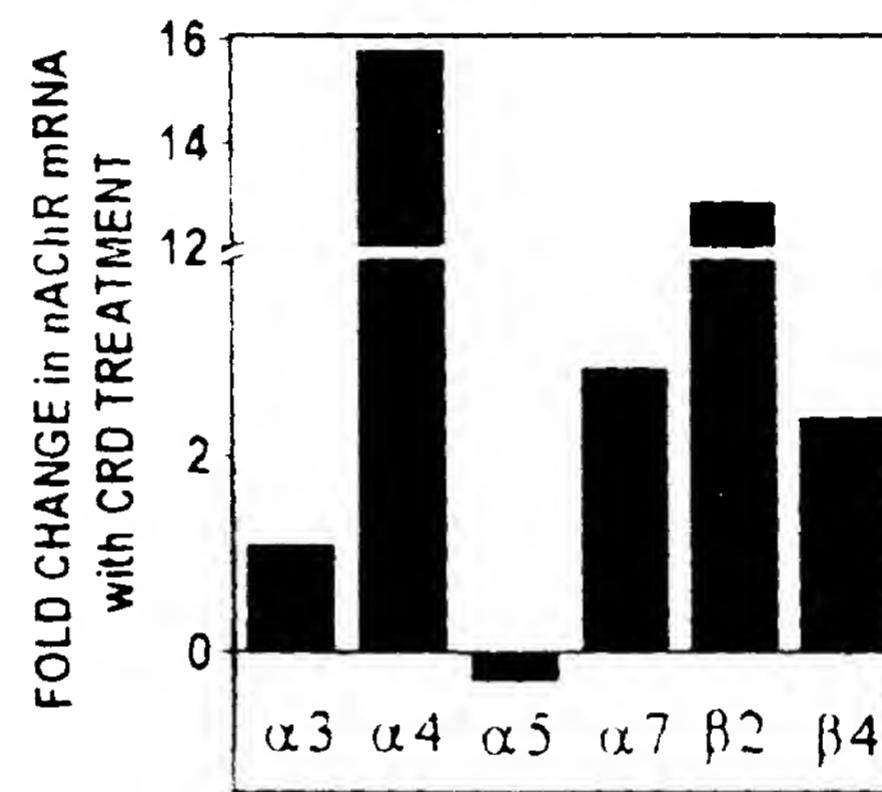
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	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 7$	$\beta 2$	$\beta 4$
VMNs <i>In vivo</i>						
(mouse)						
DEV. Δ 's; E16 vs. P0	↑ ND	↑ ↓↓	-	↑ ↓	↑ ↓	ND ND
CRD(-/-) vs. CONT						
(chick) <i>in vitro</i>						
↓ with target	↑	↑↑	↑	↑	ND	↑↑
↓ with CRD NRG	↓	↑	no	↑	ND	↑↑
MHN <i>In vivo</i>						
DEV. Δ 's	↑	↑	↑	↑	↑↑	↑↑
CRD(-/-) vs. CONT.	no Δ	↓?	↓	↓	↓?	ND
<i>in vitro</i>						
↓ with target	↑	↓	↓	↑↑	↑↑↑	↑
↓ with CRD NRG	↑	↑	↓	↑↑↑	↑↑↑	↑↑
IPN <i>In vivo</i>						
DEV. Δ 's	↑↑	↑	↑	↓	↑	↑↑
CRD(-/-) vs. CONT	"ND	↓	↓	no	no Δ	ND
<i>in vitro</i>						
↓ with input	↑	↑	↑?	↓	↑↑	↑↑
↓ with CRD NRG	↑	ND	↑?	↓↓	ND	-?↑
AMYGD <i>In vivo</i>						
↓ DEV (P0 vs. P7)	ND	↑	↑?	↑↑↑	no Δ	ND
↓ in CRD(-/-), P0 m.	ND	↓	↓?	↓?	no Δ	ND
E16 mouse <i>in vitro</i>						
↓ with input	~↑	~↑	~↑	↑↑	no Δ	ND

Mouse "in vivo" data refers to preliminary *in situ* analyses. All *in vitro* data refer to qPCR assays. - no Δ : no change in subunit levels. ND= not determined; - or ?: measurement uncertainty due to low "n" or low levels of expression. Also see Fig A2-2;

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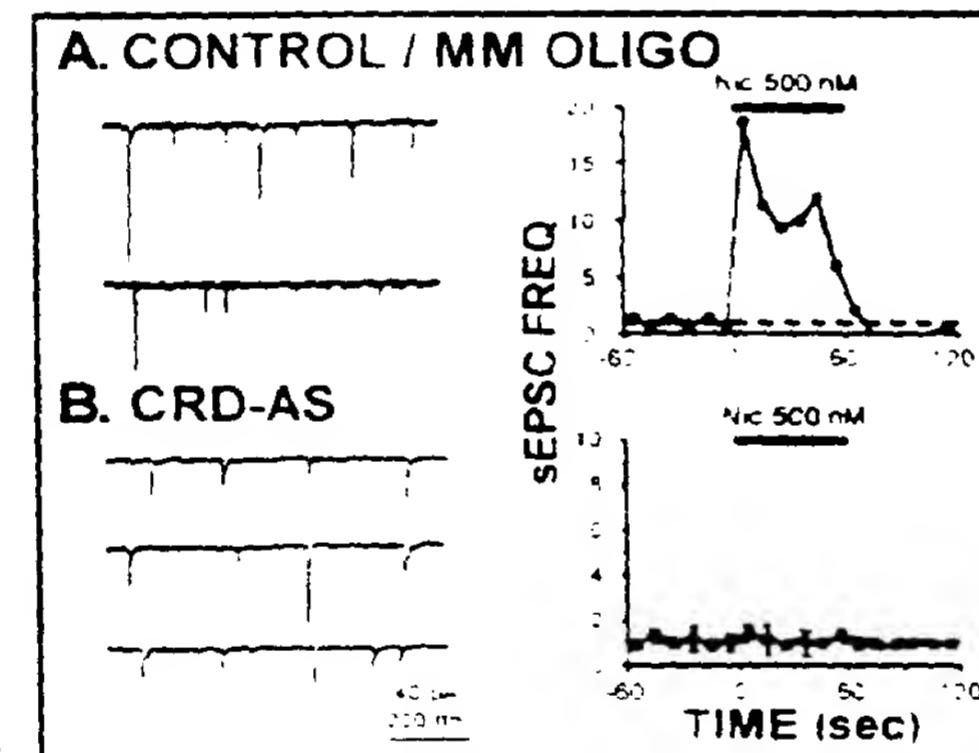
Fig A2-1: Recombinant CRD-NRG alters profile of nAChR subunit genes expressed in chick MHN neurons



nAChR subunit mRNA levels were assayed by qPCR of chick MHN neurons *in vitro*, treated (24 hrs) with rCRD-NRG or mock. The levels of subunit mRNA in mock treated neurons is set to 1.

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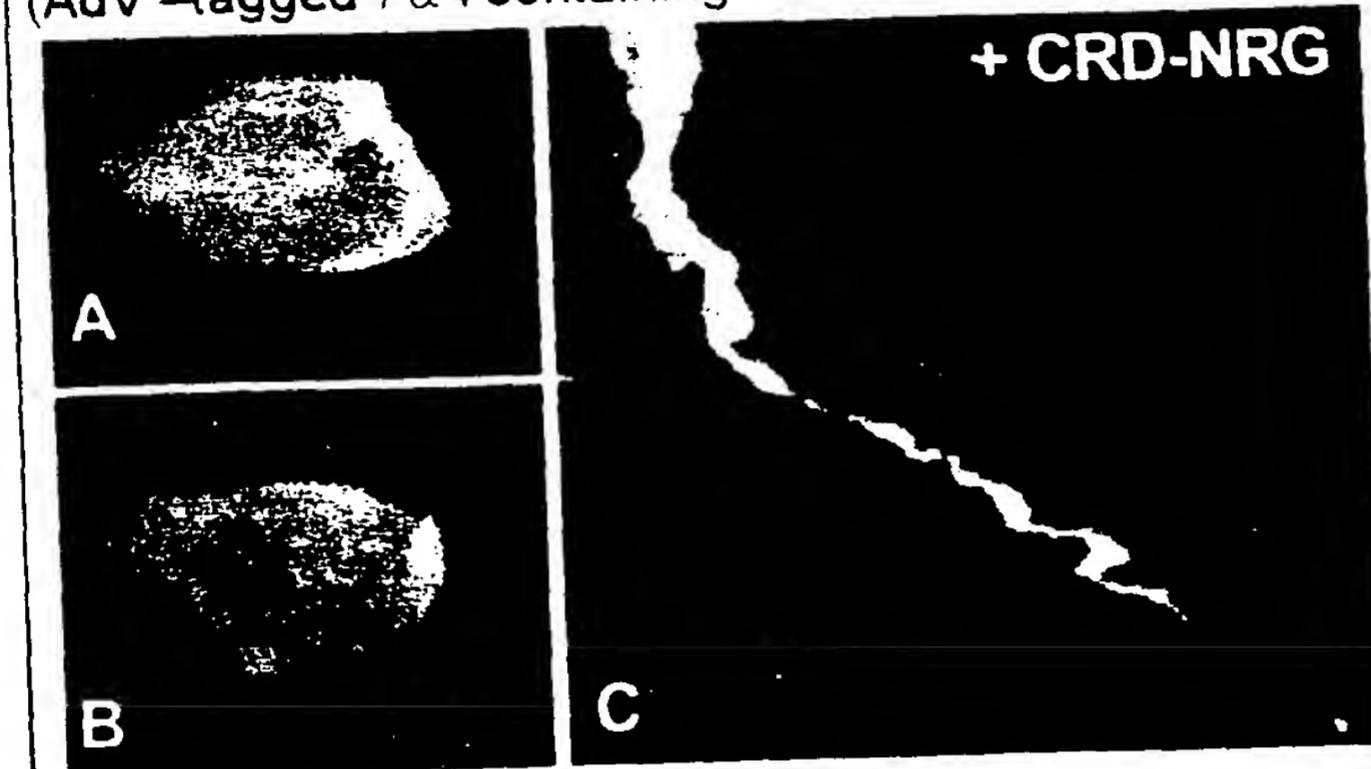
Fig A2-2 CRD-NRG signaling may be required for expression &/or targeting of presynaptic nAChRs in CNS neurons



(A).Control: presynaptic nAChRs are present at VMN - SyMp synapses, as detected by increased sEPSC frequency (synaptic facilitation) with applied nicotine. (B)Treatment of VMNs with CRD-NRG AS- (48 hrs) blocks nicotine-induced facilitation, although baseline synaptic activity (sEPSC freq.without nicotine) is unaffected. MM = mismatch (control) oligo

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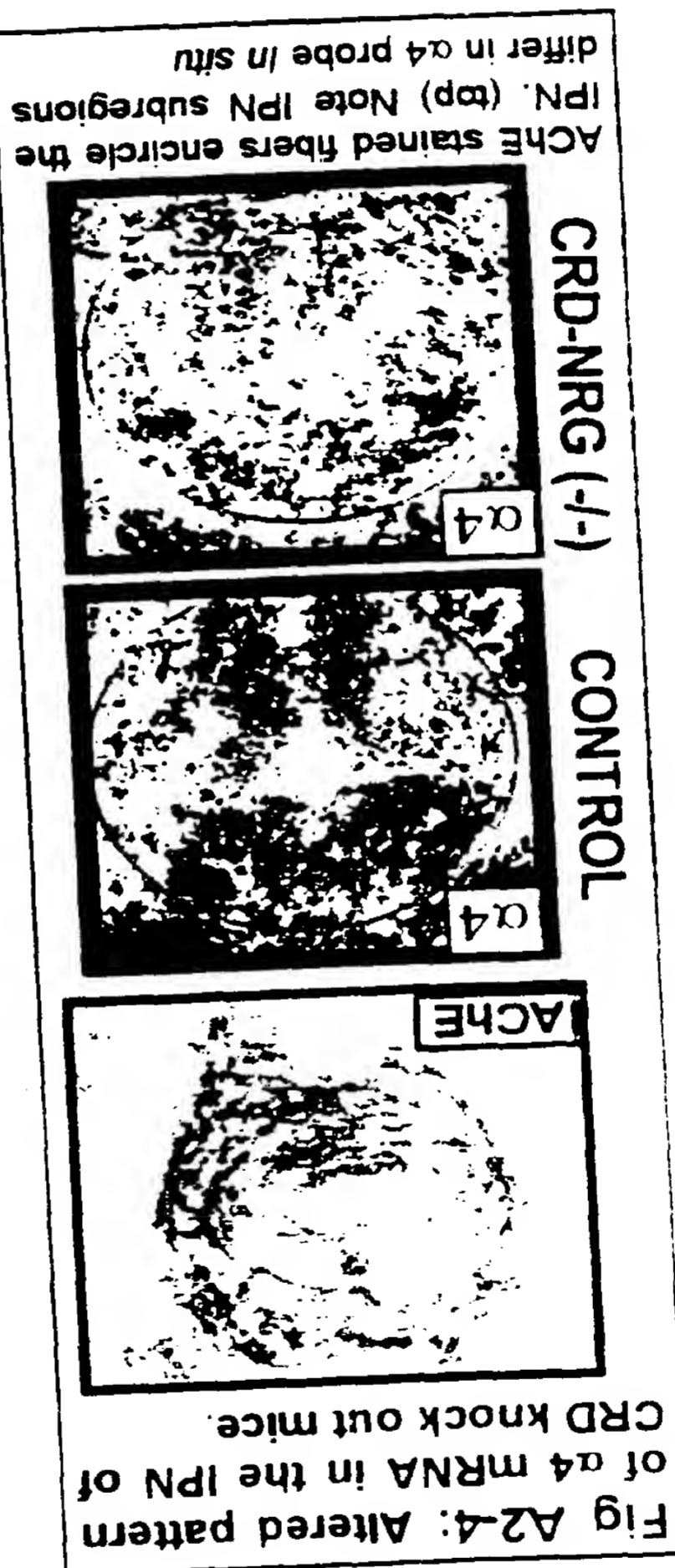
**Fig A2-3 CRD-NRG enhances axonal targeting of
(AdV -tagged) $\alpha 4$ containing nAChRs in MHN neurons**



After Adenovirus mediated gene transfer of $\alpha 4$ - FLAG in MHN,
FLAG- nAChRs are seen only on the soma of control neurons.,
whereas in CRD NRG treated (24 hrs). neurons. FLAG
nAChRs are detected in MAP-minus neurites.

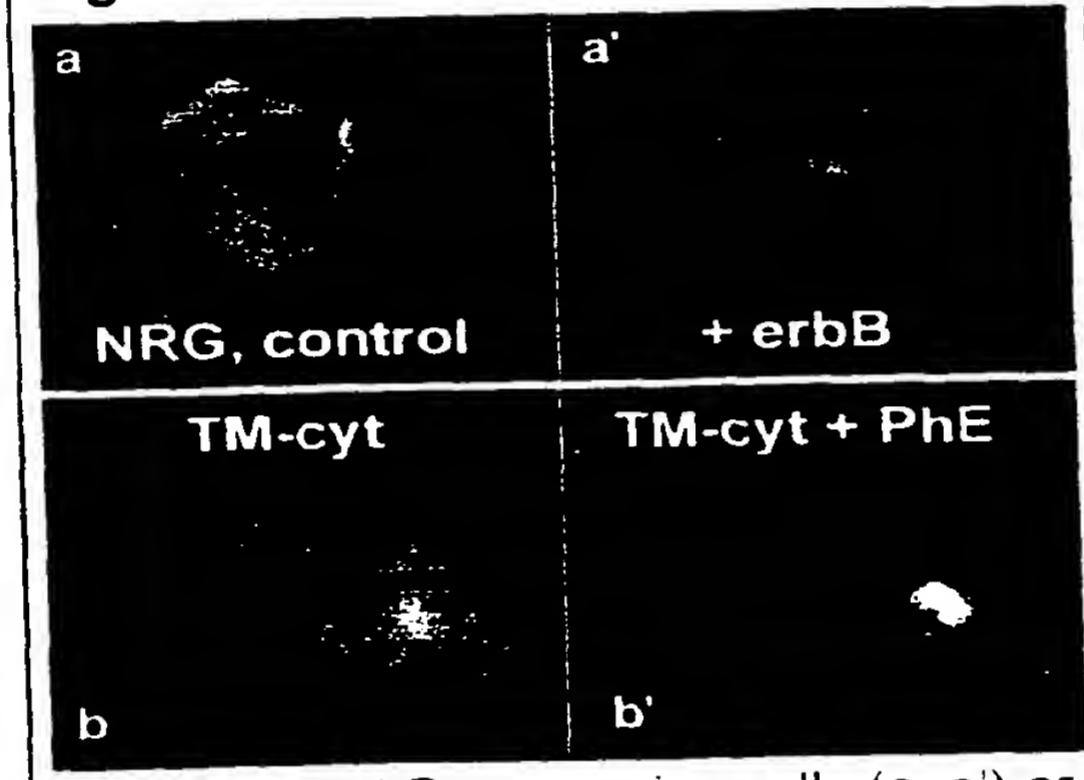
66-H150-151160

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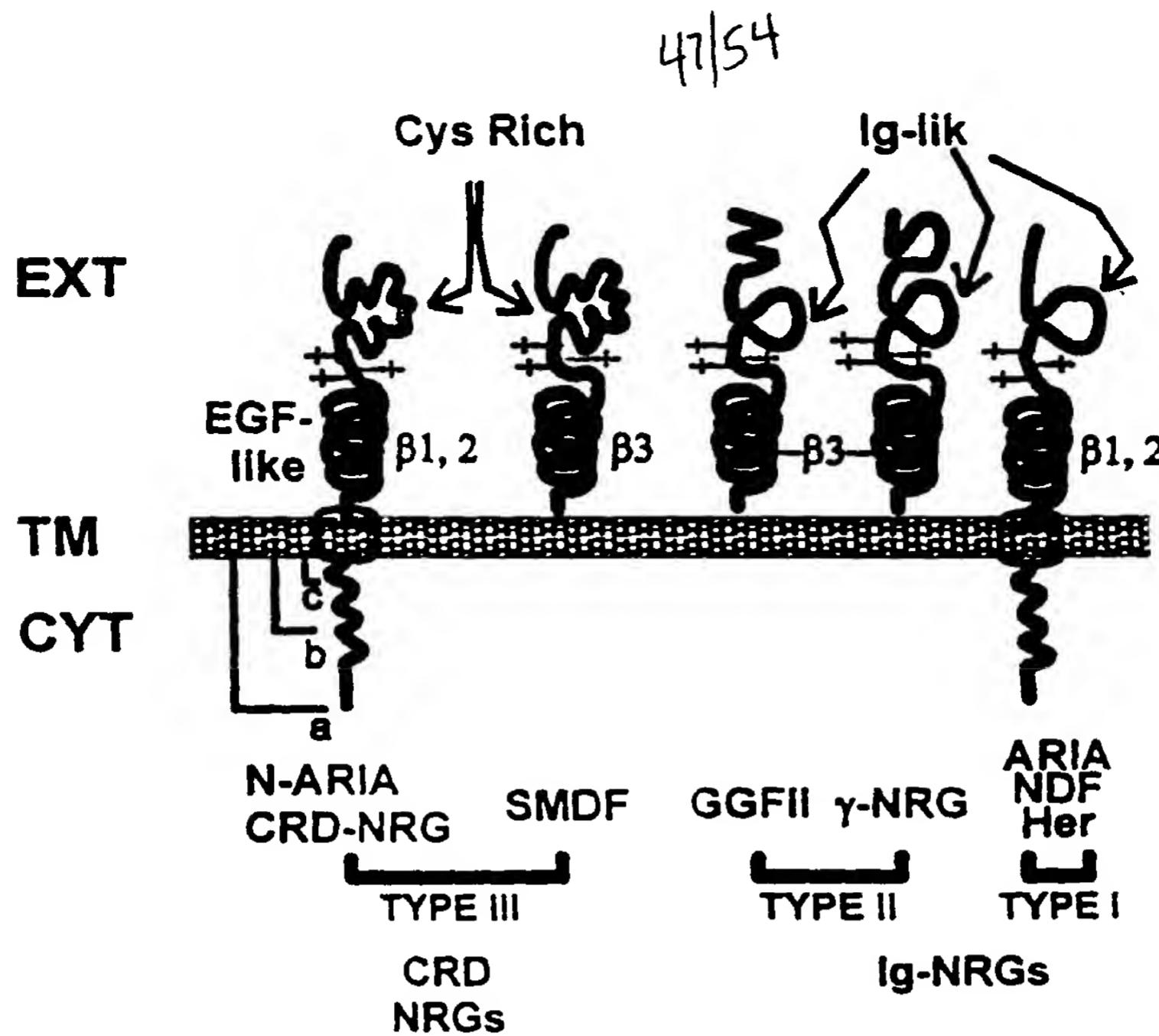


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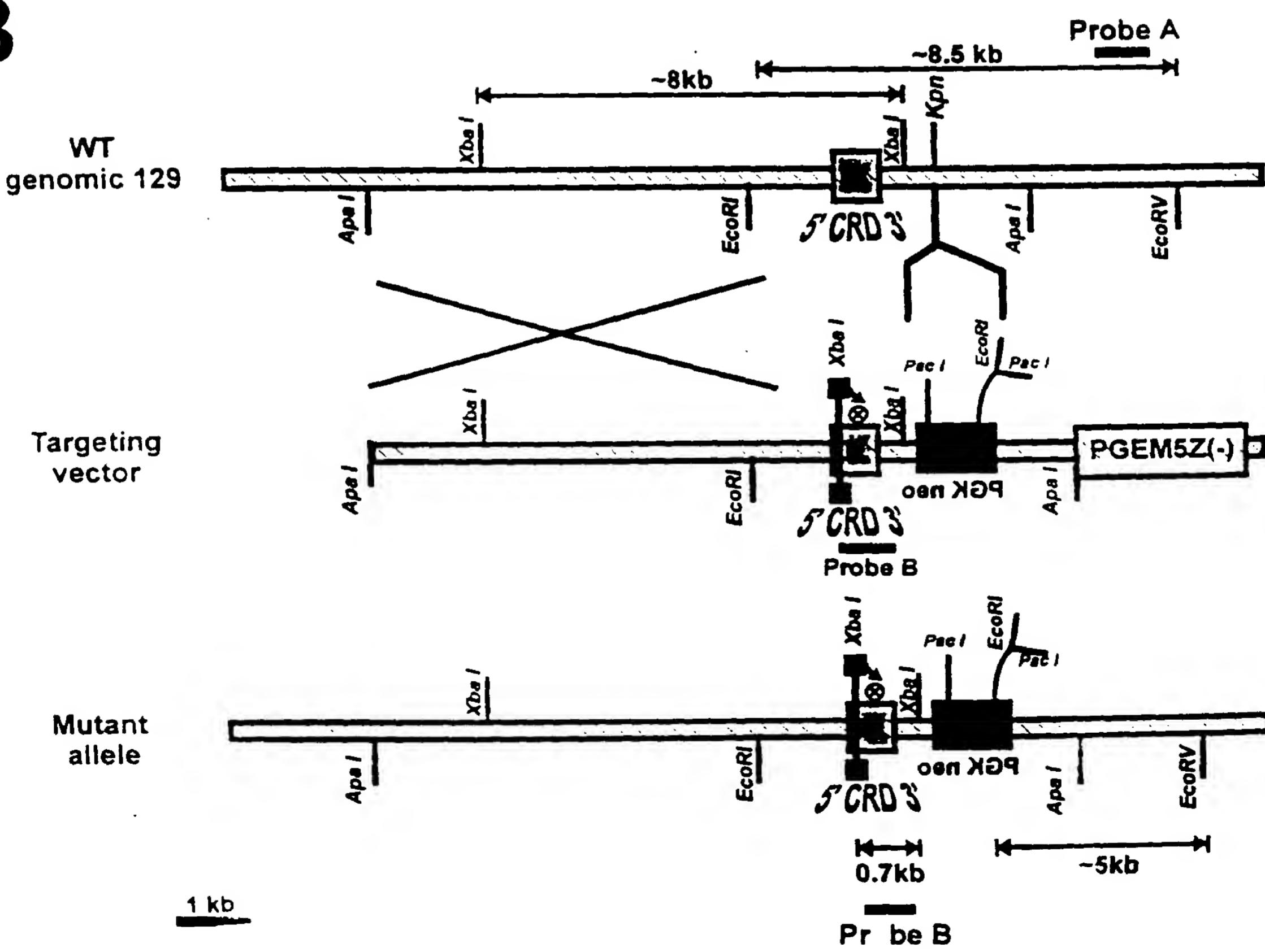
Fig A3-1 Nuclear targeting of NRG-CYT



Addition of erbB expressing cells (a, a') or activation of PKC (b,b') induces nuclear targeting of the cytoplasmic (CYT) domain from a full length NRG or a NRG transmembrane (TM) + CYT chimera

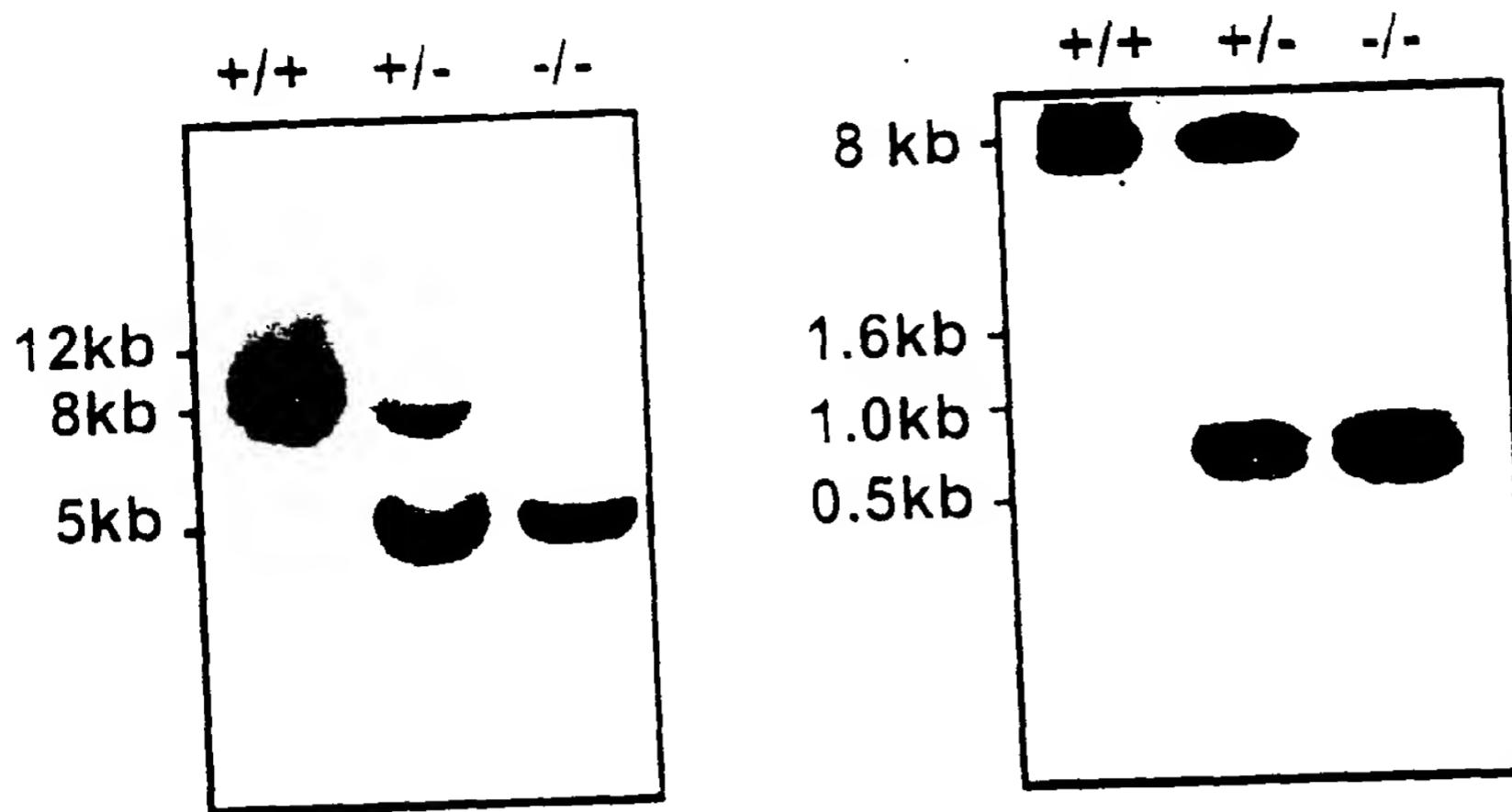
A

1 kb 5 kb 10 kb 15 kb

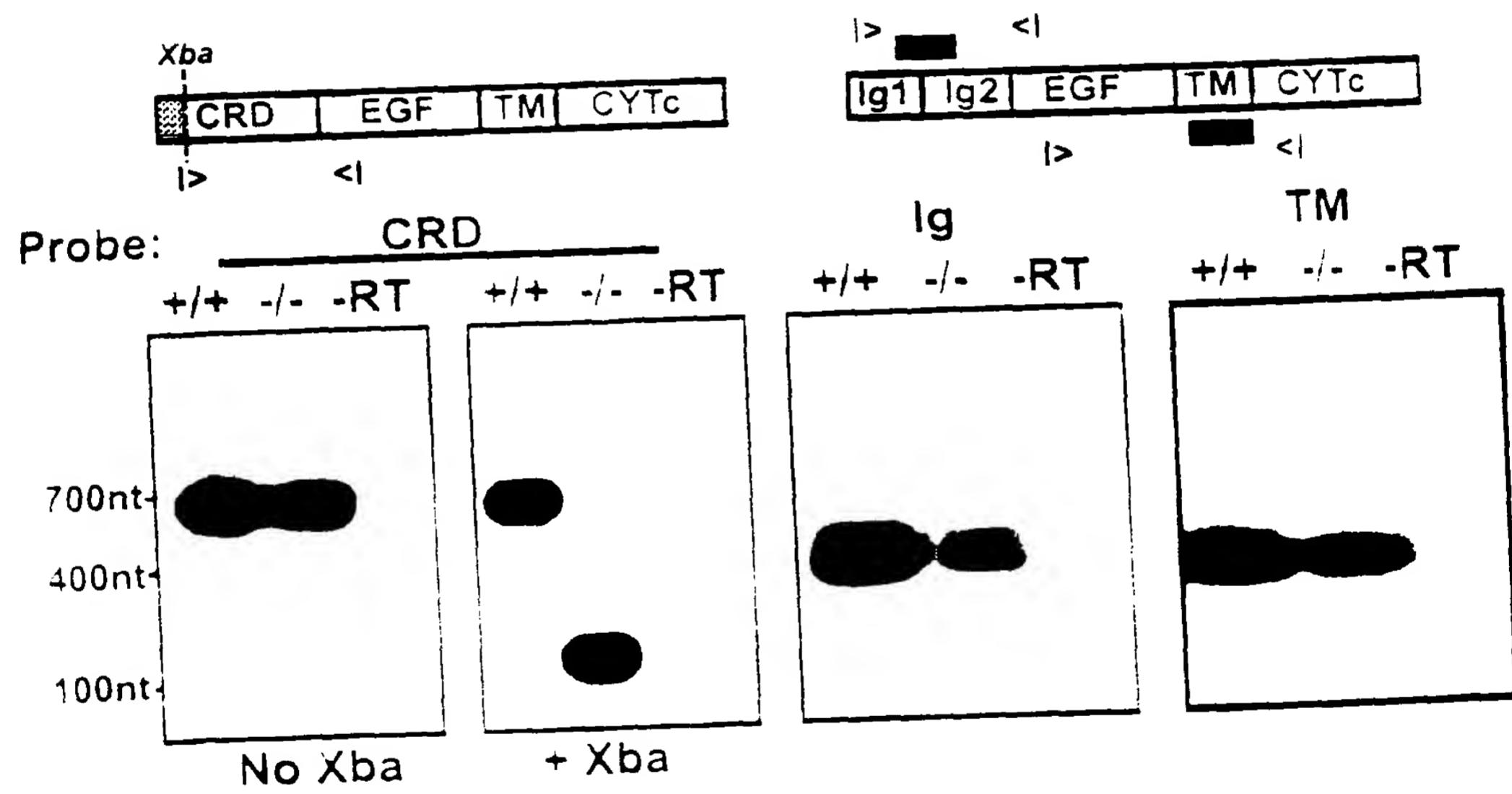
B

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C



D



E



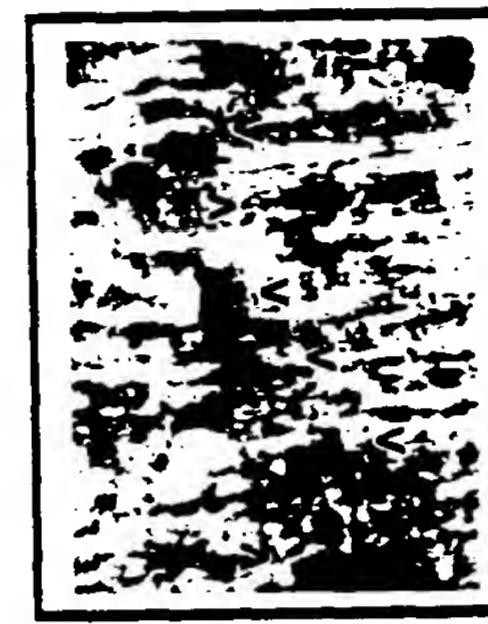
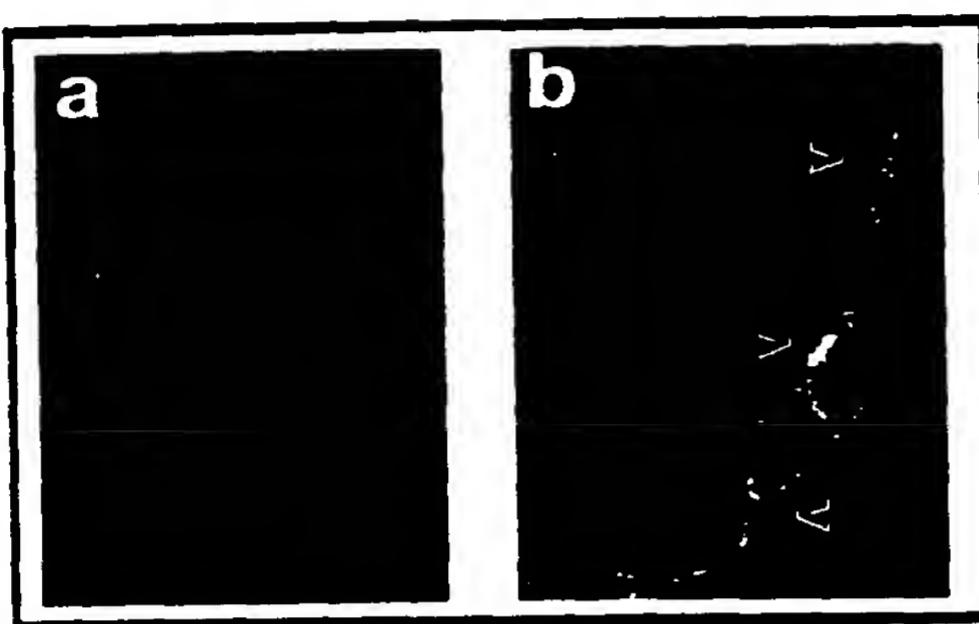
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A

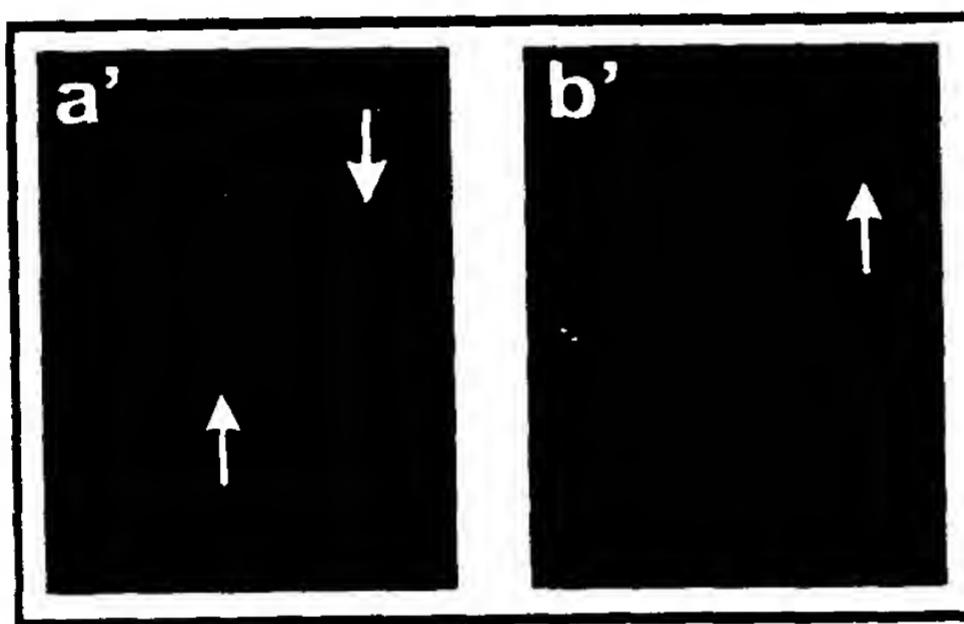
FIGURE 2A Wolpowitz et al

P0

CONTROL



CRD-NRG (-/-)



NF

+ α BgTx

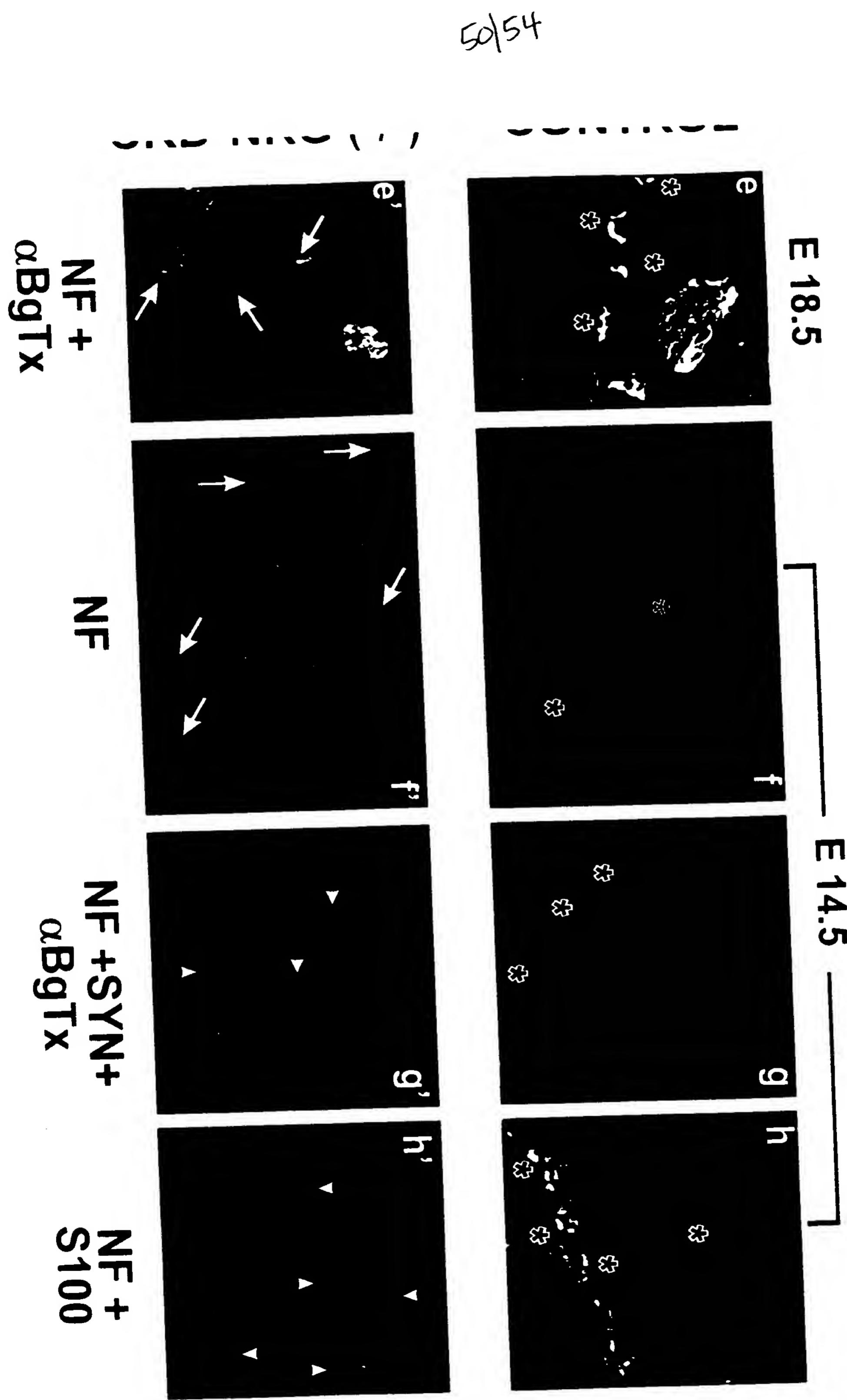


AChE



H&E

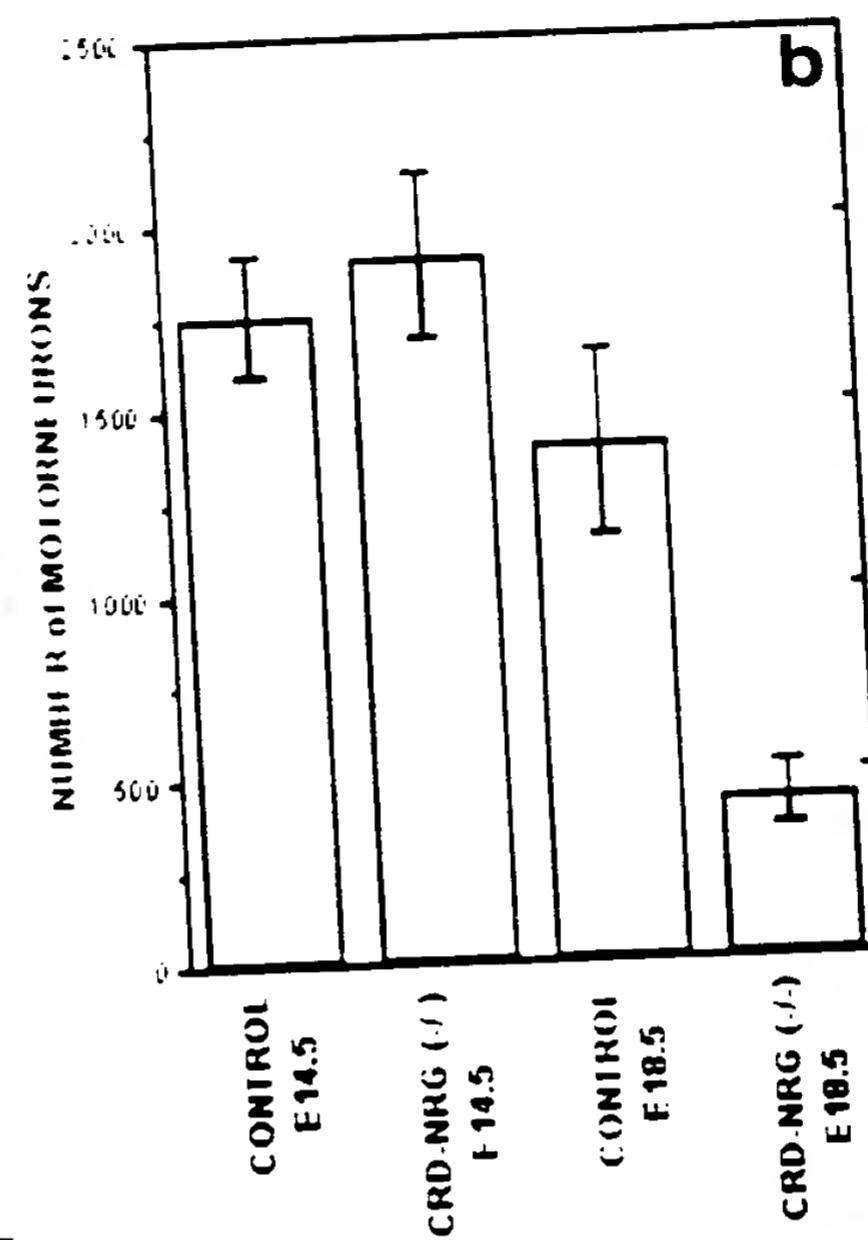
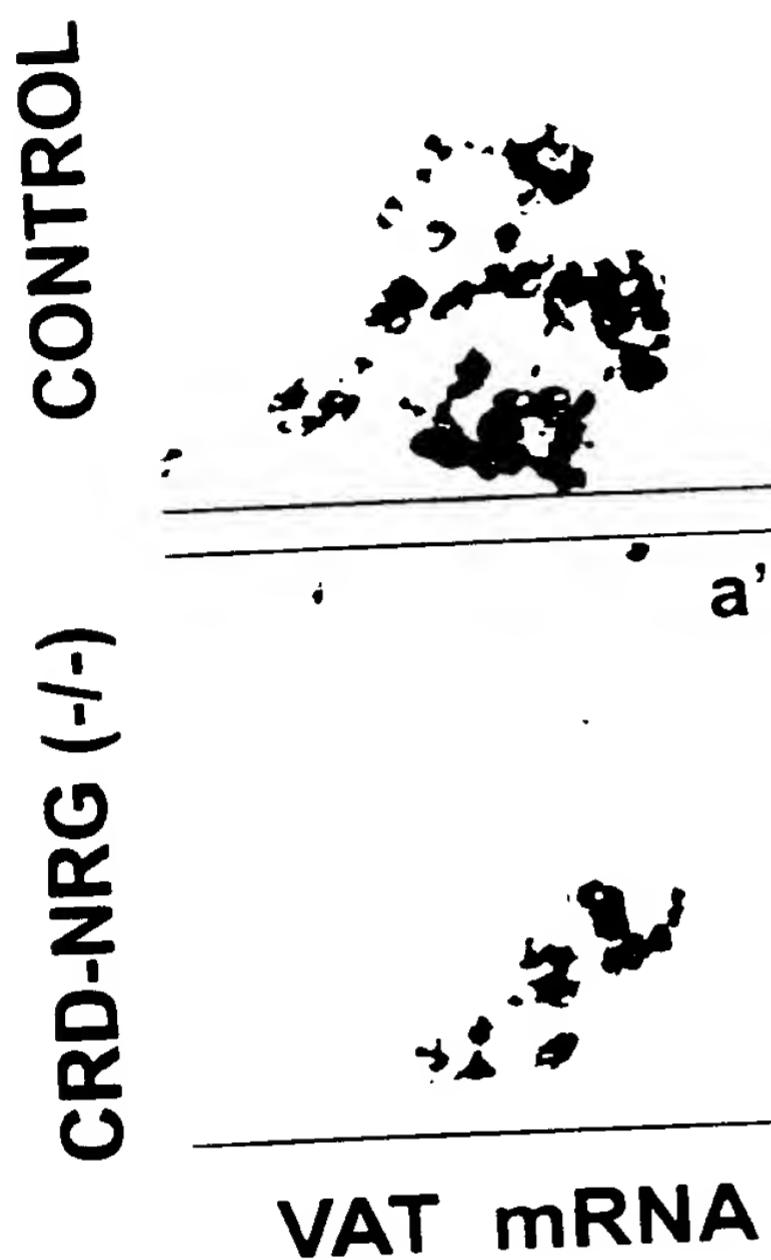
FIGURE 2B Wolpowitz et al



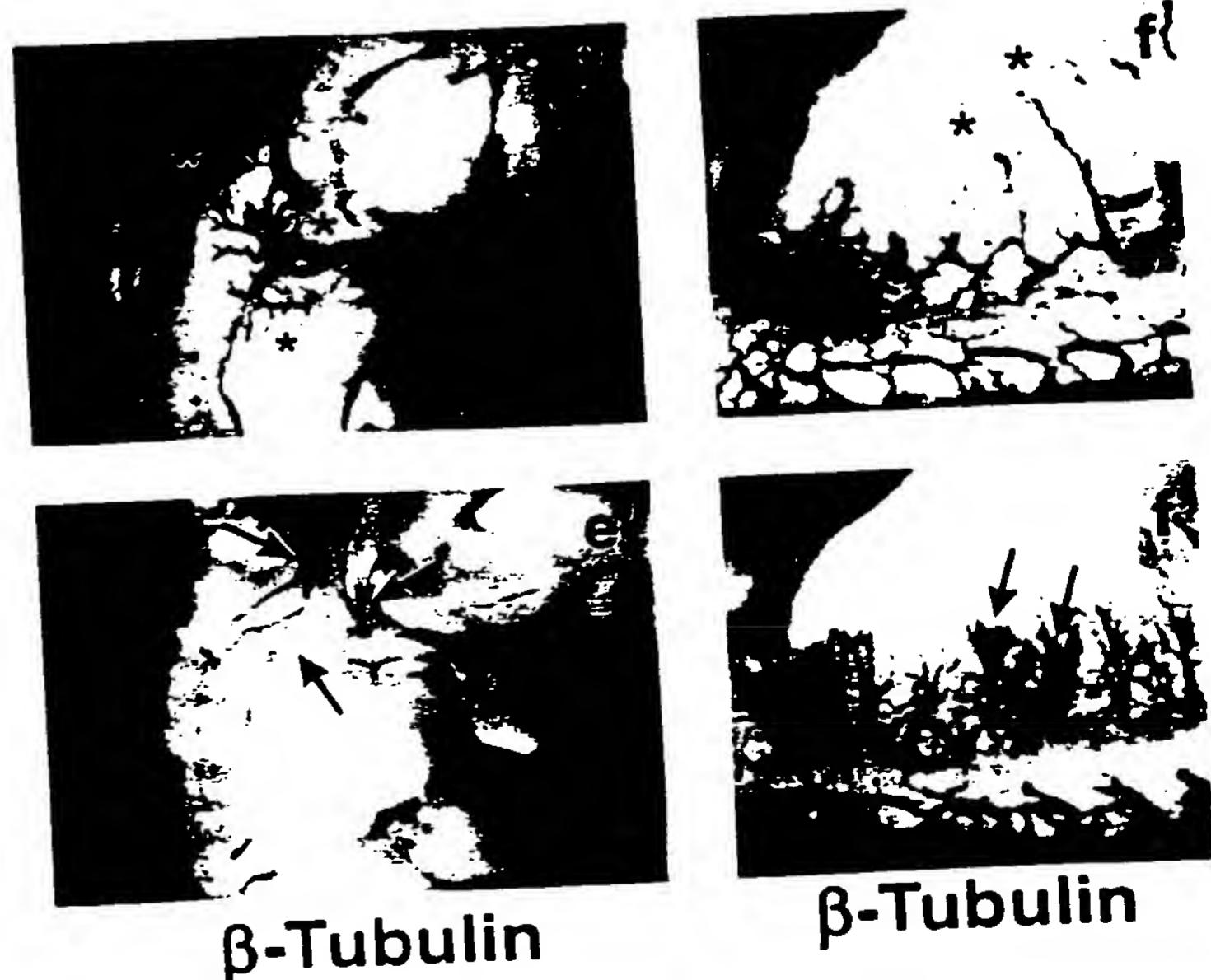
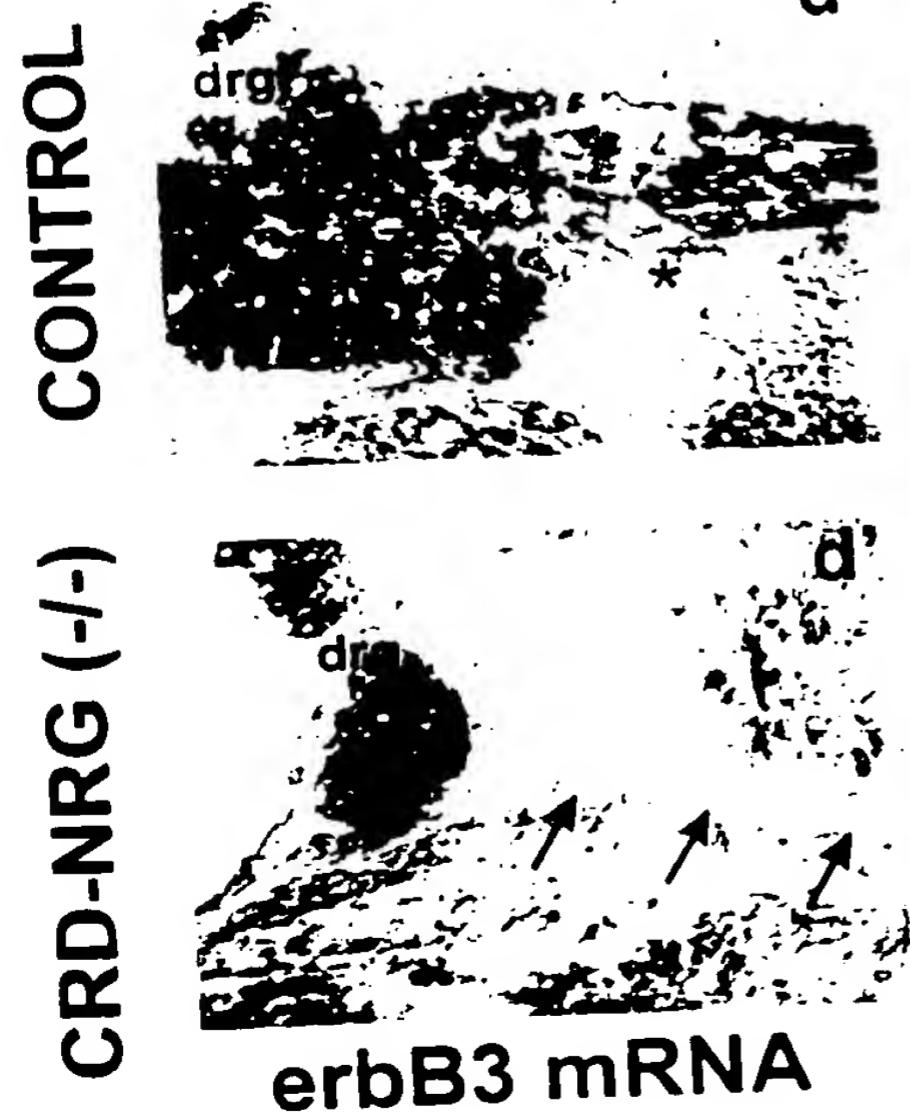
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FIGURE 3(A,B) Wolpowitz et al

A



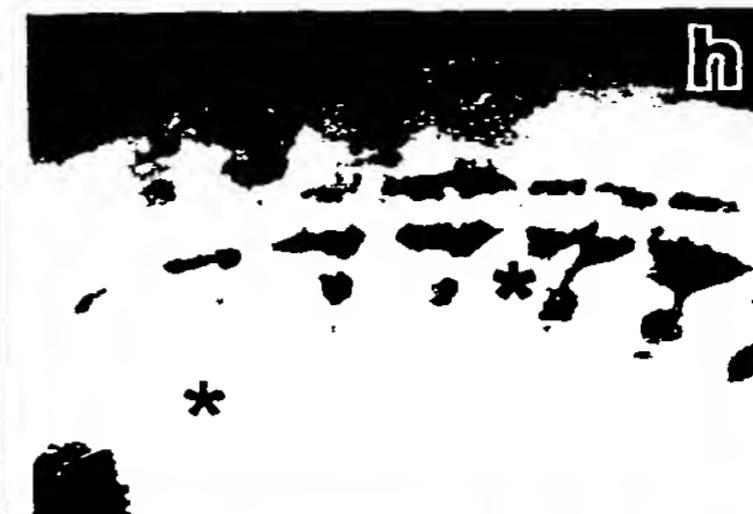
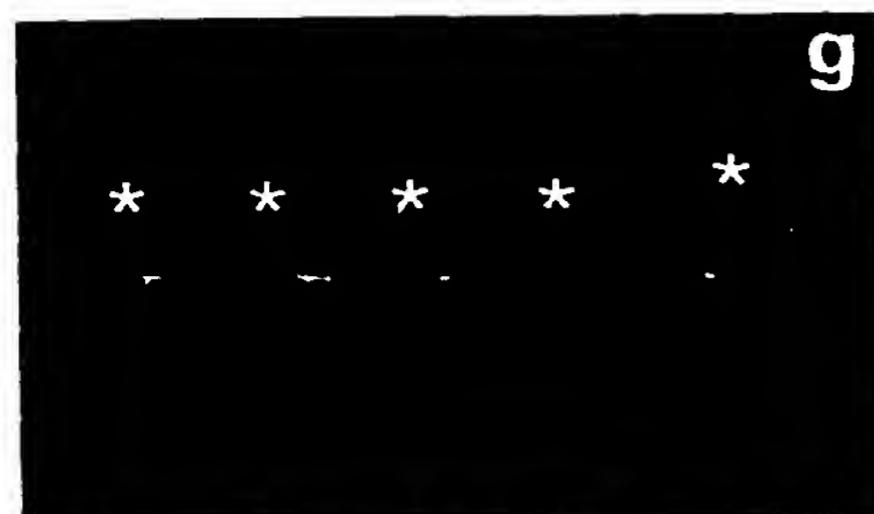
B



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C

CRD-NRG (-/-) CONTROL



VAT Ab

g'

erbB3
mRNA

VAT +
s100

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FIGURE 3(C) Wolpowitz et al.

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CN5-GANGLION

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B

FIGURE 4B Wolpowitz et al

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